ISSN 0021-9266

# Journal of Biological Education

Volume 45 Number 1 March 2011

Routledge
Taylor & Francis Group

SOCIETY OF Biology

comparison with the air, the concentration of carbon dioxide is around 200 times greater.

## Conclusions

Of course, in this case we were more interested in the problem itself and how to arrive at a solution (the thinking skills) than in the solution itself (the content). What the higher concentration of carbon dioxide in the inside of a red pepper means and why it should be so remained an open question, and is perhaps a good starting point for further inquiry-based learning in biology. Teachers and their students, however, do need a context, but very often this may detract from the ability to solve problems as giving a content label to the situation channels the thinker and closes off avenues for innovative thinking. There are in fact many content contexts for this work: gas chromatography in higher biology and chromatography in general (use and interpretation of data analysis); oxidation/reduction and gas capture/manipulation in the lower/middle years' combined science; gaseous exchange in the lower years' biology. It represents for higher-level biology students, we believe, an integrated revisionary approach of all these contexts and demonstrates that science and biology in particular should not be compartmentalised to such a degree as to inhibit thinking, and we refer back to Figure 2.

In this paper we wanted to show how, from a seemingly simple question, one can develop a complex teaching situation that leads to the realisation of teaching goals in a unique way. It shows merely one approach as an example of how to connect, link, encourage and motivate, as well as conclude and generalise. Although we have not engaged in detail in the teaching process itself, we hope that a teacher reading this paper will be able to imagine how the individual stages might be carried out, where group work would be more appropriate and where a demonstration by the teacher would suffice. Furthermore, when the question can be reformulated into a proper research question and when the lesson can be steered towards research, and how to pose questions that get pupils thinking or even lead to cognitive conflict. Finally, where the opportunities lie for a good discussion, exchange of opinions, and above all the creation of links: links between the new and the already-known, the already-taught and the forgotten, and fresh knowledge full of surprises, doubts and the old hard certainties.

## Acknowledgements

We would like to record our thanks to the Environmental Protection Laboratory at the Health Protection Institute in Maribor for performing the gas analysis free of charge and to Vojko and Mojca for their beautiful pepper fruits. We would also like to thank the reviewers for their useful comments.

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# Case study

# A multidisciplinary investigation of aquatic pollution and how to minimise it

*A. Vergnoux[a], E. Allari[a], M. Sassi[a], J. Thimonier[b], C. Hammond[b] and L. Clouzot[a]*

*[a]Centre d'Initiation à l'Enseignement Supérieur de Provence Corse, Aix-Marseille University, France; [b]Equipe de Recherche Technologique en éducation (ERTé 47), Association Tous Chercheurs, Institut de la Santé et de la Recherche Médicale, INMED, Marseille, France*

The impact of humans on aquatic systems is covered in French high schools in the 'Première' level (ages 16 to 17) by students studying economics and social sciences. We designed experiments to teach critical thinking about water pollution and how citizens can act to minimise it. The experimental session, which lasts three consecutive days, takes place in a non-profit public training centre for experimental science located within a research institute. The classroom is divided into groups of four to seven students, and each group is tutored by a PhD student. High school students are presented with results from publications on fish mutations, develop hypotheses, perform experiments and discuss their results, just as researchers do in their daily work. Students learn to ask and answer the following questions on the biological, chemical, economic and social aspects of water pollution: What are the origins of biological and chemical pollutants in an aquatic ecosystem? How can the population be made aware of aquatic pollution? How can the willingness of the local population to pay for the protection of their regional ecosystem be evaluated? At the end of the experimental session, each group of students prepares a poster presentation and discusses with an external expert.

**Keywords:** aquatic pollution; wastewater treatment plant; sustainable development

## Introduction

We designed the present practical course for French high school students in 'Première' level (students aged 16 to 17), option Economics and Social Sciences. It can also apply to fifth-grade students as long as they have basic knowledge on ions, molecules, cells (bacteria in particular), and DNA and its replication.

Our objectives are to teach students to think critically on a scientific question: here, aquatic pollution, with its multiple facets in biology, chemistry, economy and sociological sciences. Much like scientists, students are instructed to review scientific publications, elaborate hypotheses, design experimental protocols and perform experiments, and discuss experimental results. Analysis of the content of the posters enables determination of whether the objective is met.

Water pollution is suitable as it allows young citizens to critically get in touch with its various facets including pesticides, phthalates, alkylphenols, natural and synthetic hormones which induce fish feminisation (European workshop, Weybridge, UK, 1996) or fish mutations such as the two-mouth fish found in Alberta near an industrial hydrocarbon release site (LSN TVA, Quebec, 19 August 2008). The efficiency of wastewater treatment plants (WWTPs) meant to purify industrial and municipal wastewaters before their release into aquatic environment are notoriously insufficient, hence the importance of illustrating and debating this issue with young students.

### *Educational methods*

The practical lasts three consecutive days. The students are gathered into groups of four to seven, each

Corresponding author: C. Hammond, INMED UMR 901 Inserm, 163 route de Luminy, 13273 Marseille Cedex 9, France. Email hammond@inmed.univ-mrs.fr, tel 33 4 91 82 81 00 / 10, fax 33 4 91 82 81 01

*Journal of Biological Education* ISSN 0021–9266 print/ISSN 2157–6009 online © 2011 Society of Biology
http://www.informaworld.com
DOI: 10.1080/00219266.2011.537839

supervised by a tutor who is a PhD student. The session (Figure 1) begins with a three- to four-hour group discussion centred on the same two slides on fishes (Figure 2). Tutors are trained neither to ask nor to teach data, but to answer students' questions and reframe the discussion. Students are encouraged to organise their questions and with the support of the tutor to design suitable experimental protocols to test these issues. At that point, the tutor establishes the following contract with the students:

> you have thought about a lot of possible questions on the subject, but it is impossible to investigate all of them. Therefore, I suggest that you test these particular points concerning chemistry, or microbiology, or economics or social aspects in order to put together a complete story addressing the general issue.

In the following two days they learn to read and follow a protocol, design and conduct experiments, quantify results, discuss, and interpret and draw (or not) conclusions. In parallel, they prepare a mini-poster summarising their questions and experimental work.

Relying on the mini-posters, each group then explains to the other groups the topics investigated, results obtained and the conclusions drawn. This both enables promotion of the oral presentation and reinforces *multidisciplinarity* and group cohesion, since each group can grasp the tools used to respond to the question. The tutors' roles are to conduct the session and ensure that each group fully understands the work done by other groups. At that point, students are sorted into three (or more) new chimera groups with students from other groups (Figure 1). They design the final posters that will summarise and synthesise the questions and experiments performed by all groups to provide a complete picture including the chemistry, microbiology, economics and social issues. An external researcher is then invited for an hour-long discussion with the students. Photographs of the posters are taken for future evaluation.

We evaluate the posters on the following criteria: (i) whether the title is representative of the poster's content; (ii) structure and general organisation (introduction, order of sections, subtitles for sections, intermediate conclusions, general conclusion); (iii) the images chosen to illustrate the experiments and results; and (iv) the presence of scientific mistakes, if necessary (we always suggest corrections). Students' comments on their posters are also evaluated; one example of these includes the presence of a hypothesis-driven approach in their explanations, rather than simple recapitulation of the results.

## Results

### *Observations, questions and hypotheses*

Tutors show two slides (Figure 2) about phenotype modifications of different species of fish living in two types of environments subjected to the influence of human activity. On the first slide, there is a short text, extracted from a scientific journal (*Sciences en brèves* 62, April 2004; www.cemagref.fr). The second slide, from an informational website, shows a fish with two mouths caught in an Alberta lake. Students gradually come up with the following questions: What could be the source of these mutations? What is released into the water that could lead to such changes? In general, students try to understand the cause of phenotype changes in fish and the origin of the pollutants. They rapidly identify the wastewater treatment plants (WWTPs) as a pollution source. They ask how WWTPs operate to understand the reasons why they may pollute aquatic ecosystems. They hypothesise two types of pollution, chemical and biological, and

<table>
<tr><th></th><th></th><th>Microbiology</th><th>Chemistry</th><th colspan="2">Economic / Social</th></tr>
<tr><th>Day</th><th>Schedule</th><td>group 1</td><td>group 3</td><td>group 4</td><td>group 5</td></tr>
<tr><td rowspan="2">Monday</td><td>9h<br>12h</td><td colspan="4">Discussion</td></tr>
<tr><td>14h<br>17h</td><td rowspan="2">Petri dishes / PCR solutions</td><td rowspan="2">Spectro-photometry</td><td>Metaplan<br>Economics</td><td>Economics<br>Metaplan</td></tr>
<tr><td rowspan="3">Tuesday</td><td>9h<br>12h</td><td rowspan="2">PCR / Respirometric tests</td><td rowspan="2">Spectro-photometry</td><td>Metaplan<br>Economics</td><td>Economics<br>Metaplan</td></tr>
<tr><td>12h<br>14h</td><td colspan="4">Poll</td></tr>
<tr><td>14h<br>17h</td><td>Macroscopic observations</td><td>Colorimetric tests / pH</td><td>Metaplan<br>Economics</td><td>Economics<br>Metaplan</td></tr>
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**Figure 1. Planning**

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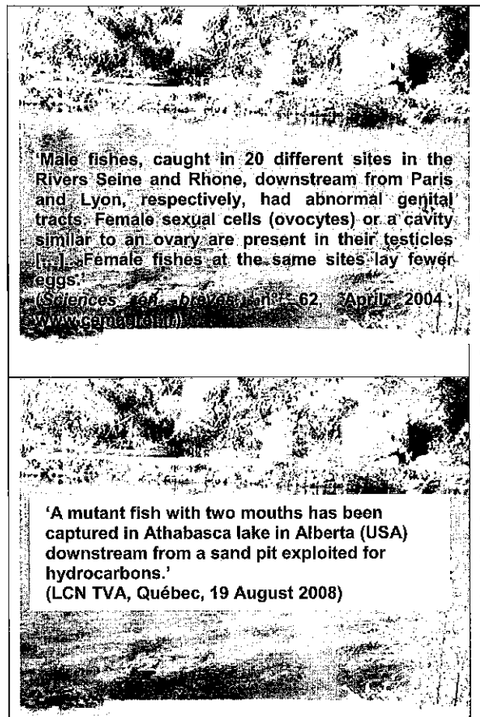



Students rapidly hypothesised that water pollution could be at the origin of these observations. They made a list of possible sources of pollution: industrial, agricultural (fertilizers, pesticides...) and human rejections. They said that urine may contain drugs, medications and hormones that could feminise fishes.

How do these pollutants arrive in rivers? Through wastewater. They pointed out that wastewater is treated, so they asked 'how do WWTPs work?' They studied with tutors the different phases of domestic wastewater treatment: primary and secondary treatments and the role of bacteria.

How to test whether a suspicious molecule found in a river came from the WWTP? They had the idea of taking different samples of water and analysing them. They summarised their work:

**Observations**: river fishes may display strange phenotypes.

**Hypothesis**: water pollution is responsible for them.

**Experiments:** measurement of pollutants upstream, at the level and downstream from the WWTP.

**Figure 2. Observations and students' remarks**
Photograph by Ludiwine Clouzot.

propose that chemical pollution comes from insufficient purification of wastewater, whereas biological pollution directly results from the biological wastewater treatment and the release of these micro-organisms into the environment.

The question of the consequences of human activity and WWTPs on the natural aquatic environment becomes the central theme of the brainstorming session. They discuss discharges, the difference of quality between drinking water and water released in rivers, systems for wastewater treatment and more generally on pollution sources and their impact on ecosystems. To evaluate the imbalance created by humans, and their impact on environment and humanity, on a short-, medium- and long-term scale, tutors emphasise the concepts of 'system' and its key notions (interdependence and equilibrium). Some students have difficulty understanding the link.[2] To conclude and summarise, tutors ask students to draw a diagram containing a WWTP located on a river to explain the spatial sequence of water treatment process and pollutant discharge in aquatic environments (Figure 3).

Students usually disregard the economic and social aspects of pollution. Tutors ask them to think about solutions to minimise pollution by WWTPs. Since this will cost money, they propose a poll to estimate how much the local population is willing to pay to increase protection of the regional ecosystem and to create slogans to increase public awareness on aquatic pollution.

### *Experiments*

The general aim of the experiments is to analyse the microbiological and chemical differences between three different water samples:

- Upstream: a river sample upstream of a WWTP effluent;
- Effluent: a river sample at the level of the WWTP effluent;
- Downstream: a river sample downstream of the WWTP effluent.

### *Microbiological approach*

The microbiological experiments address the questions: What types of bacteria are present in the three water samples? Are they quantitatively or qualitatively different? What is the biological impact of WWTPs on rivers? More precisely, are there bacteria released by WWTPs and polluting aquatic ecosystems? (We explain to students that there is a biological treatment of wastewater and they quickly

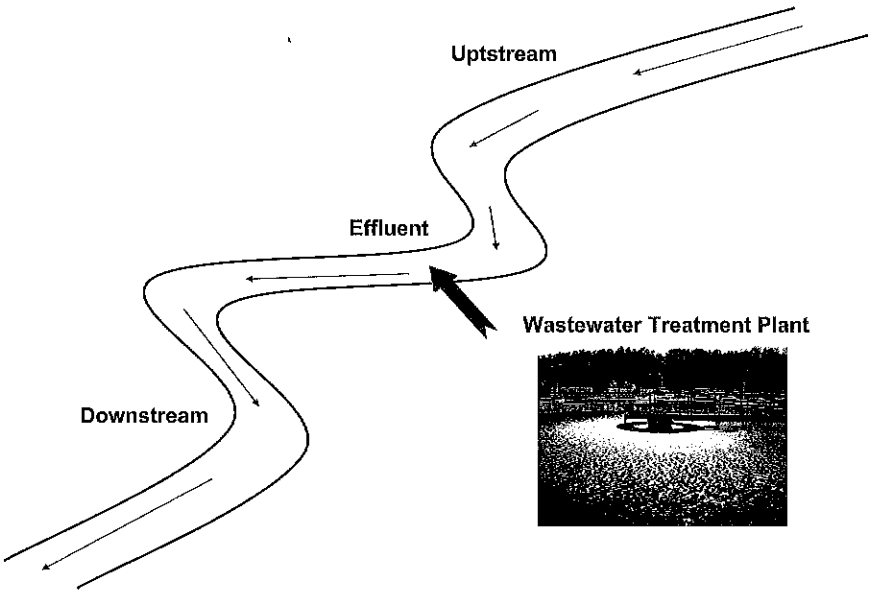



**Figure 3. WWTP's release into the aquatic environment**
Photograph by Ludiwine Clouzot.

understand that biomass has to be removed prior to being released into the environment.) Experiments enable identification of the phenotypes and metabolism of bacterial colonies grown in Petri dishes using macroscopic and microscopic observations, respirometry, and polymerase chain reaction (PCR) techniques (Appendix 1).

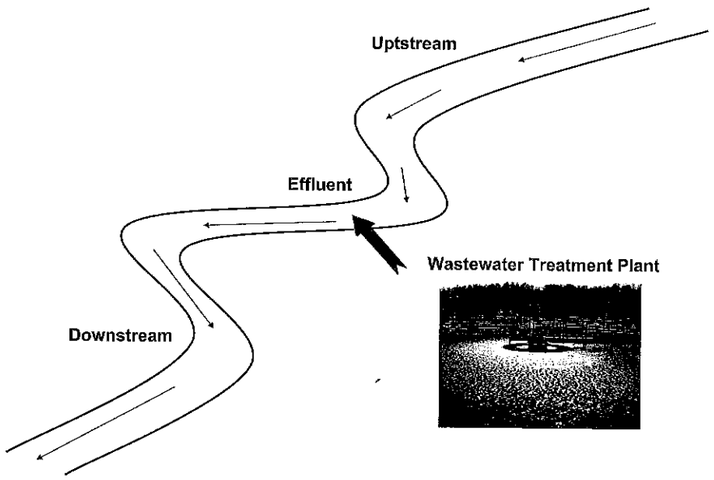
Bacterial colonies grew on Petri dishes corresponding to the effluent and the downstream samples (Figure 4a) but not (<10) on those corresponding to upstream from the WWTP effluent. The colonies in the Petri dishes of the effluent sample were more numerous than in the downstream sample. Colonies were similar in all the Petri dishes: middle sized (<3 mm), circular, round, smooth, opaque and cream-coloured identified as S colonies (Figure 4b). They were stick-shaped, moving, but without a specific movement or a specific grouping mode. They were identified as anaerobic because they developed in the bottom of the tubes (Figure 4c) and PCR analysis enabled identification of *Escherichia coli* (E.c.) and 'universal bacteria' (U.B.). E.c. was used as an indicator of faecal contamination and U.B. as an indicator of contamination from WWTP biomass. Electrophoresis migration showed that neither E.c. nor U.B. were present in pure water. U.B. were present in all three samples but E.c. was not detected in the samples (Figure 4d). Students planned the positive and/or negative controls for all their experiments. The upstream sample was used as the control for macroscopic and microscopic observations. Pure water was used as a negative control and suspensions of E.c. and *Citrobacter* were used, respectively, as E.c.- and U.B.-positive controls in PCR experiments. Students concluded that the WWTP effluent contains a high amount of bacteria and probably brings them to the river. These bacteria seemed to be macroscopically and microscopically similar in the effluent and the river and to have similar types of metabolism. Among these bacteria, *E. coli* was not detected. *E. coli* is a necessary digestion bacterium for warm-blooded animals but may provoke infections outside of the intestine. It has the ability to survive for brief periods outside the body, making it an ideal test of faecal contamination. It was absent from effluent and downstream samples, as expected if wastewater has been properly treated.

## Chemical approach

What types of ions are present in the three water samples? Are they quantitatively or qualitatively different? Does the WWTP effluent modify the chemical composition of rivers? Experiments conducted enabled to compare the concentrations of $Cu^{2+}$, $Fe^{2+}$, $Fe^{3+}$, $K^+$, $NH_4^+$, $H^+$, $PO_4^{2-}$, and $Cl^-$ ions in the three water samples using colorimetry, photometry and pH measurements (Appendix 2). No conclusions were drawn about the concentrations of $K^+$, $Fe^{2+}$, $Cu^{2+}$ and $NH_4^+$ since the concentrations were below the threshold for detection (Table 1). In contrast, results obtained showed that the effluent discharged $Fe^{3+}$ in

**Table 1. Chemical characterisation of the three water samples**

| | Upstream | Effluent | Downstream |
|---|---|---|---|
| pH | 7.73 | 8.17 | 7.73 |
| $Cu^{2+}$ (mg $l^{-1}$) | 0.1 | 0.1 | 0.1 |
| $K^+$ (mg $l^{-1}$) | < 200 | < 200 | < 200 |
| $Fe^{2+}$ (mg $l^{-1}$) | < 0.1 | < 0.1 | < 0.1 |
| $Fe^{3+}$ (mg $l^{-1}$) | < 0.1 | 1 | 0.1 |
| $NH_4^+$ (mg $l^{-1}$) | < 2.6 | < 2.6 | < 2.6 |
| $PO_4^{2-}$ (mg $l^{-1}$) | 6.02 | 3.3 | 5.08 |

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| $PO_4^{2-}$ (mg $l^{-1}$) | 6.02 | 3.3 | 5.08 |



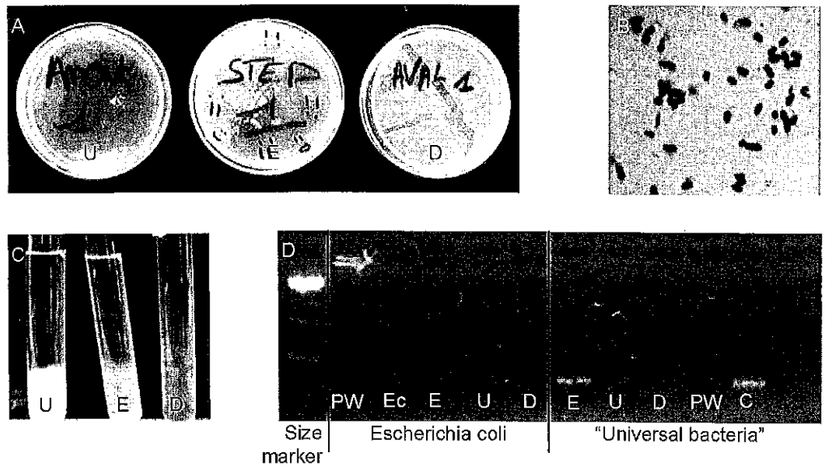


**Figure 4. A. Bacterial development in Petri dishes from the three samples (Amont, upstream; step, effluent; aval, downstream). B. Microscopic examination of bacteria from a colony of the WWTP effluent. C. Results of respirometry tests. D. PCR results**
Notes: U, upstream; E, effluent; D, downstream; PW, pure water; Ec, *Escherichia coli* suspension; C, *Citrobacter* suspension. Photographs taken by Jean Thimonier.

### Economics approach

Two groups designed an economic poll to estimate the willingness of the interviewed population to pay for the ecosystem preservation. This includes three steps: (i) creation of the economic poll (Table 2); (ii) interview of the population; and (iii) economic analysis of the result (Appendix 3). Each group interviewed approximately 12 people (76 people, total). One group interviewed undergraduate students and professors (heterogeneous population) from the campus and the other group interviewed professors and PhD students (homogeneous population) in laboratories. Students performed basic statistics (Figure 5a). Men and women were present in equal proportion. Most (78%) of the population was unaware of aquatic pollution and is in favour of environmental projects improving water purification by WWTPs. The graphical representations explaining the willingness to pay (WTP) and expressed as: $WTP = f\ (gender)$ (1) and $WTP = f\ (age)$ (2) are shown in Figure 5b. Students concluded from the two graphs that gender had no influence on the willingness to pay, whereas the age of the interviewed population did since young students were less willing to pay for ecosystem preservation when compared to professors or PhD students. We explained to them that age in the interviewed population also indicated level of education. The homogeneity of the interviewed population was due to the population from which subjects were drawn, which was a purely academic population (Bateman 1993).

### Social approach

Tutors used a specific educational tool named Metaplan® (Appendix 4) derived from psychological studies and management tools, which teaches discourse, representations, ideas and meanings. This tool taps into the creativity of a group and is based on the participation of each member as well as interactions between members (Borcier 2006). A metaplan is relevant for collecting ideas and building a shared representation of the world (Godet 2004). This technique gives students the opportunity to address the issue and make connections between the different scientific fields covered by the experimental session. The reflection part, termed the awareness campaign leads to the creation of slogans (the 'active' part) that are written and discussed by the whole group. Results of this last session are given in Table 3.

### Oral and poster presentations

On the final day, each group of students presents the results of their workshop to the others. During this oral session many of the students who had performed microbiological or chemical experiments wrongly concluded that WWTP effluent 'directly' provokes 'mutations' of river fishes. We explained to them that they did not observe mutations in the two slides (Figure 1), but only strange phenotypes, and that bacteria or pollutants detected from the studied WWTP cannot be directly linked to mutations (if they exist) without additional

**Table 2. The questionnaire created by high school students**

| | Questions | Responses |
|---|---|---|
| 1 | Age | |
| 2 | Gender | |
| 3 | Professional occupation | |
| 4 | Awareness of the risk | Yes: 1<br>No: 0 |
| 5 | Interest in this environmental and economic matter | Yes: 1<br>No: 0 |
| 6 | Your opinion about the project led by your regional government | Scale from 1 (no interest) to 10 (very good) |
| 7 | According to you, should the regional government concentration on other topics?<br>If yes, which ones | Yes: 1<br>No: 0<br>Comments |
| 8 | The government has to increase local taxes for 10 years in order to conduct the project. Here is a payment table in Euros, select the preferred amount | Refer to the payment table |
| 9 | Does the location of your residence have an influence on your willingness to pay? | Yes: 1<br>No: 0 |
| 10 | Suppose that you are living near this WWTP, would this increase your willingness to pay?<br>If yes, how much could you pay? | Yes: 1<br>No: 0<br>Refer to the payment table |
| 11 | Suppose that the environmental project led by the government needs more money than initially believed, would you pay more?<br>If yes, how much would you accept to pay? | Yes: 1<br>No: 0<br>Refer to the payment table |

Payment table

| | | | | | |
|---|---|---|---|---|---|
| 0 | 1.5 | 3 | 4.5 | 6 | 7.5 |
| 9 | 10.5 | 12 | 13.5 | 15 | 18.75 |
| 22.5 | 26.5 | 30 | 33.75 | 37.5 | 41.25 |
| 45 | 48.75 | 52.5 | 60 | 67.5 | 75 |
| 150 | 300 | 450 | 600 | 750 | Other |

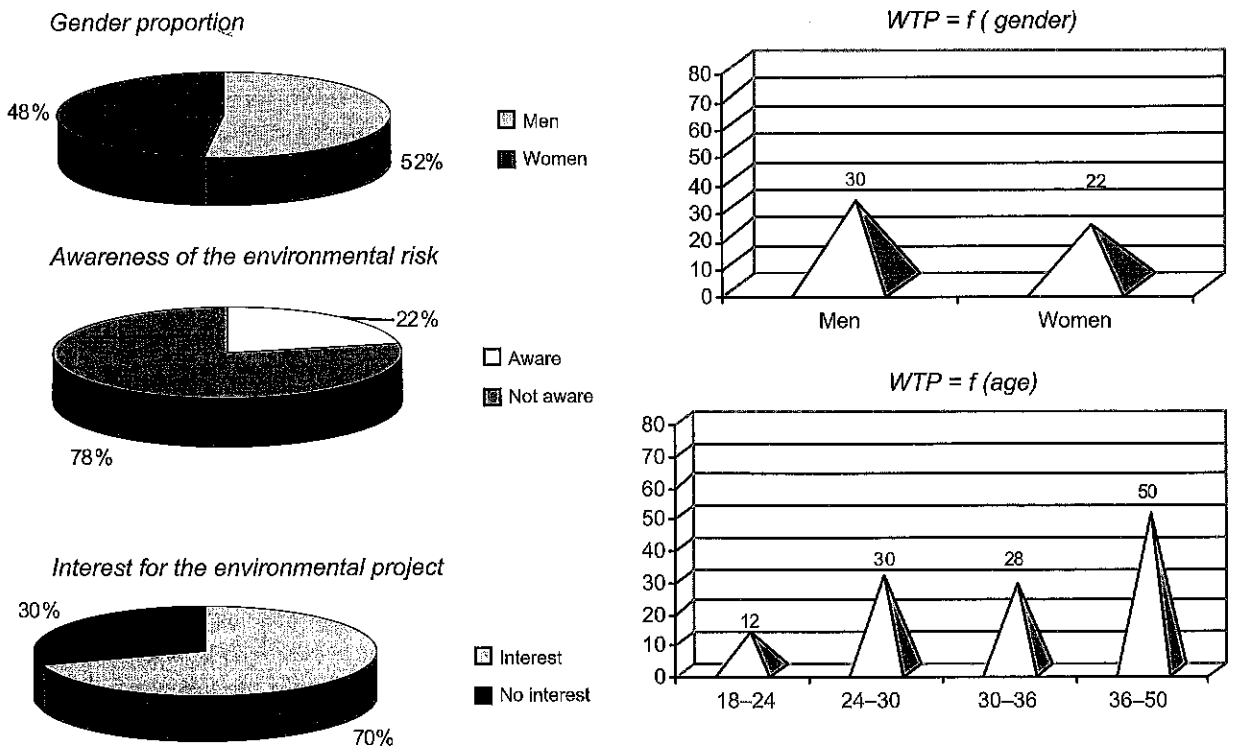


**Figure 5. Economical results from polls. A. Graphical representation of the willingness to pay. B. Basic statistics explaining the willingness to pay valuation for the ecosystem preservation**

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| | Questions | Responses |
|---|---|---|
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| 8 | The government has to increase local taxes for 10 years in order to conduct the project. Here is a payment table in Euros, select the preferred amount | Refer to the payment table |
| 9 | Does the location of your residence have an influence on your willingness to pay? | Yes: 1<br>No: 0 |
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Payment table

| | | | | | |
|---|---|---|---|---|---|
| 0 | 1.5 | 3 | 4.5 | 6 | 7.5 |
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| 22.5 | 26.5 | 30 | 33.75 | 37.5 | 41.25 |
| 45 | 48.75 | 52.5 | 60 | 67.5 | 75 |
| 150 | 300 | 450 | 600 | 750 | Other |

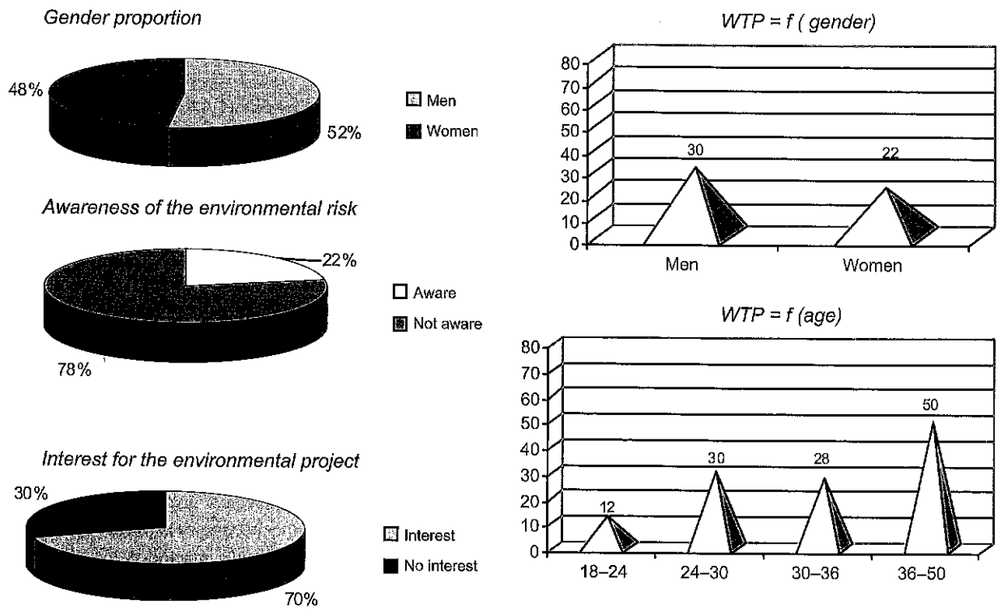


**Figure 5. Economical results from polls. A. Graphical representation of the willingness to pay. B. Basic statistics explaining the willingness to pay valuation for the ecosystem preservation**



**Table 3. Slogans created by two groups of students**

| Slogans (Group A) | Slogans (Group B) |
|---|---|
| 'Gene mutations: the beginning of our extinction … Take Action!' | 'Think small to get big results: every gesture counts!' |
| 'A small gesture for man, a big step forward for life!' | 'Consider the difficulties of the future without existing solutions' |
| 'The effort of today … well-being forever!' | 'The future is now being built … and if we change our habits?' |
| 'Fish forgotten, a link broken … Preserve the ecosystem' | 'Detergents damage people and the environment!' |
| 'Abuse of cleaning products, fish in danger!' | 'The ecosystem, I love and respect it!' |
| 'Prevent mutations (in fish) … Reduce your consumption!' | 'Stop fish feminisation, find solutions' |

experiments. It was a good opportunity to make them practise critical thinking and learn how false conclusions may be drawn from correct results.

All of the four posters created by the class had a title, and three clearly indicated the question asked: 'WWTP are needed to treat waste water but what are their limitations, and what is the position of society on this problem?' 'Do WWTP pollute the aquatic environment?' In the third, a fish asked the question: 'Why is my natural environment polluted and how can it be fixed?' Considering general organisation, all posters were clearly subdivided into two to three sections but only one had synthesised the work as: (1) Problems; (2) Possible solutions. The others had a categorical organisation directly reflecting the different groups ((1) Microbiology, (2) Chemistry, (3) Solutions). Only one clearly indicated the comparison between upstream, effluent and downstream water samples. All of the figures, diagrams and pie charts shown were relevant. Figure 6 shows a poster which showed innovative organisation.

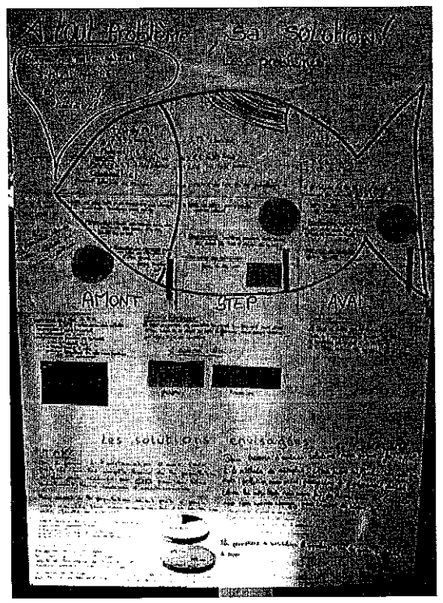


**Figure 6. Poster presentation**

## Discussion

This educational experience is rewarding and we achieved our aims. Indeed, oral presentations and posters helped students understand the way researchers work to obtain and verify a result, rigorous methods of analysis and interpretation, the importance of scientific discussions, the concept of a multidisciplinary approach, and the advantage of team operation.

We encountered several problematic issues. Students were too numerous in groups of six to seven: four seemed ideal. Students were also shocked by the photos of strange fishes, and the conclusions of students on their microbiological and chemical experiments were often incorrect (see above). The point in the exercise at which students are analysing their results and drawing conclusions is critical. Tutors must take the time to explain why it is incorrect to directly conclude from their experiments that bacteria and $Fe^{3+}$ are responsible for fish mutations. They must ask students to carefully summarise their observations, think about the additional experiments that should be performed to reach (or not) that conclusion, how to identify a correct or incorrect argument, and how to build a conclusion from objective data. The challenge is to make students understand the problem at its most essential level.

In general, students reported that the economic and social approaches fairly well complemented the microbiological and chemical approaches to the WWTP problem. Although the metaplan exercise was far removed from scientific protocols, it was a good opportunity to tackle the 'reflection–awareness' part of this project. Tutors took the opportunity of the poster creation phase, when students were in their final process of deliberation, to help them understand the problem more broadly. Thanks to these discussions, topics gradually transcended the single issue of mutant fish. We also emphasised the concept of 'ecosystem'.

Working as a team, so that the expertise of each contributed to a common purpose is an exercise that requires multiple intellectual and 'social' abilities. Tutors saw the emergence of some conflicts blocking group progression, whereas others got along well and quickly progressed. Students had to face, often for the first time, the difficulties and advantages of working in

a group. Overall, tutors were impressed by their ability to synthesise and present their data. Poster presentation was particularly rewarding for the students. To be interviewed and congratulated by an external informed and experienced figure was a privileged moment for them.

## Notes

1. Tous Chercheurs is a non-profit organisation. The teaching staff consists of postdoctoral researchers. http://www.touschercheurs.org
2. For example, a student said, when we were talking about mutant fish: 'Madam, I don't care about fish, I don't eat fish!'

## References

Bateman, I.J. 1993. Valuation of the environment, methods and techniques: Revealed preference method. In *Sustainable environmental economics and management, principle and practice*, ed. R.K. Turney, 1. London and New York: Belhaven Press.

Borcier, M. 2006. *Fiche méthodologique d'animation metaplan*. Paris: INPES

Godet, M. 2004. *La boîte à outils de prospective stratégique. Cahier du LIPSOR* 5: 1–114.

# Appendix 1: Identification of the bacteria present in river samples near a wastewater treatment plant

## 1A. Techniques: bacterial culture seeding and observation under light microscopy

### *Aim*

To characterise bacterial colonies by macroscopic examination after they have multiplied in Petri dishes. Describe colonies by size, shape, appearance ... in order to identify the family and even the species to which they belong.

### *Protocol*

NB: Unless otherwise stated, use filtered water only.

| Samples |
|---|
| Water upstream, at the level of, or downstream of a wastewater plant |

- Put 200 µl of the water sample in the middle of the Petri dish filled with agar.
- Spread out the drop with a curved glass pipette spreader.
- Close the Petri dish and place it in the incubator at 37°C for 12–24 h.
- Take out the Petri dish and observe the macroscopic characteristics as explained above.
  =>Do not open the Petri dishes when you are far from a Bunsen burner.

## 1B. Techniques: microscopic observation

### *Aim*

To identify the family and species of bacteria present in water samples, through observation by light microscopy of their shape, grouping, and movement characteristics.

### *Protocol*

NB: Unless otherwise stated, use filtered water only.

| Bacterial clones |
|---|
| To be sampled from petri dishes |

- Sample a clone with a sterile curved glass pipette and place it in a tube filled with 100 µl water milliQ.
- Add 2µl of methylene blue (Jeulin, france). Bacteria become violet.
- Vortex the tube or agitate until the clone dissociates.
- Take a drop from the tube and put it in the middle of a coverslip.
- Put a glass slide alongside the drop, and cover the sample gently, making sure not to trap any air bubbles.
- Observe under a light microscope, with low intensity light and no oil. Place the slide onto the microscope stage underneath the objectives. Lower the objective. Close the diaphragm. Raise the stage to within 1 mm of the objective and begin observation using the eyepieces. Focus by lowering the sample away from the objective, while looking in the eyepieces, and adjusting the fine focus until the image is clear and in the plane of the eyepiece micrometer.
  => If the cells are too dense and cannot be observed properly, dilute the bacterial solution.
- When observing with the 100× objective, add a drop of oil.

a group. Overall, tutors were impressed by their ability to synthesise and present their data. Poster presentation was particularly rewarding for the students. To be interviewed and congratulated by an external informed and experienced figure was a privileged moment for them.

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|---|
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|---|
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  => If the cells are too dense and cannot be observed properly, dilute the bacterial solution.
- When observing with the 100× objective, add a drop of oil.



## 1C. Techniques: respirometry test 'fluid thioglucolate medium' (FTM)

### *Aim*

To characterise the bacteria present in water samples using analysis of bacterial respiration.

### *Protocol*

NB: unless otherwise stated, use only filtered water.

| **Bacterial clones** |
|---|
| Take samples of bacterial clones derived from the three different water samples |

| **Culture medium FTM** |
|---|
| Pour 29.7g of dehydrated medium into 1 litre of water<br>Heat at 100°C to dissolve the powder<br>Fill tubes with culture solution<br>Sterilise in autoclave at 121°C for 15 min |

- Sample each clone with a glass pipette.
- Introduce the pipette into the tube filled with FTM medium.
- Lower with a spiral movement.
- Raise, but do not take the pipette out of the medium.
- Lower again.
- Raise.
- Put the tube into the incubator at 37°C for 12 h.
- Identify the region of the tube where the bacteria developed.

## 1D. Techniques: PCR and electrophoresis in agarose gel (identification of bacteria in water samples)

### *Aim*

To amplify a DNA sequence specific to *Escherichia coli* in order to detect their presence in water samples using PCR (polymerase chain reaction).

### *Materials*

Control bacteria: *E. coli* and *Citrobacter*
Primer (Invitrogen)

- Universal
  Forward: 5′–CAGAGTTTGATCCTGGCTCAG–3′
  Reverse: 5′–CTGCTGCCTCCCGTAGGAGT–3′
- Specific to *E. coli*
  Forward: 5′–TAAGCTCGGGAGGAATGATG–3′
  Reverse: 5′–GCCAGGTGAGTTTCTCTTGC–3′

PCR mix
1 µl forward primer + 1 µl reverse primer, universal or specific (final concentration 10 µM)
12.5 µl GoTaq Green Master Mix 2X (Promega)
8.5 µl $H_2O$ DNAse free

### *Methods*

#### *Solutions*

- Filter 20 ml of the water sample to test using a Millipore membrane (0.22 µm). Then take out the membrane with swizzles and place it in a clean Eppendorf tube.
- Add 200 µl milliQ water and resuspend the deposits present on the filter membrane.
- Transfer this solution to test in a clean Eppendorf tube.

#### *DNA amplification*

- Add 2 µl of the water to test or of the control solution (*E. coli*, *Citrobacter* or milliQ water) to 23 µl of the PCR mix.
- Place tubes in the thermocycler (Mastercycler personal, Eppendorf) and programme as follows:

| | **Temperature** | **Duration** |
|---|---|---|
| 1 cycle | 94°C | 5 min |
| 25 cycles | 94°C<br>58°C<br>72°C | 30 s<br>30 s<br>30 s |
| 1 cycle | 72°C | 2 min |

#### *Analysis of PCR products*

Samples are analysed with electrophoresis in 1% agarose gel in a TAE solution (Tris–acetate 40 mM, EDTA 1 mM).

### *Note*

1. Fluid thioglycollate broth is a reducing medium that contains compounds that react with molecular oxygen, keeping the free levels low. It also contains the indicator dye reazurin, which turns pink in the presence of oxygen. Since oxygen is present at the surface of the medium, the upper layer is usually pink whereas the dye is colourless in the remainder of the tube. Agar is also included

in this medium to give it a semi-solid consistency. This prevents the movement of inoculated organisms. Fluid thioglycollate broth has something for every microbe. Strict aerobes will grow only at the top of the tube, strict anaerobes only at the bottom, facultative and aerotolerant anaerobes throughout the tube, and microaerophiles somewhat below the surface.

# Appendix 2: Identification of the ions present in river samples near a wastewater treatment plant

## 2A. pH measurement

### *Aim*

To measure the pH of three different water samples.

### *Materials*

- A pH meter and its electrodes.
- Solutions for calibration at pH 9.2, 6.86 and 4.01.

#### *Standardisation*

*First step*

Pour the solution pH = 6.86 into the glass beaker (electrodes must be well submersed in the solution). Then follow the instructions corresponding to the pH meter you use.

*Second step*

Remove electrodes and rinse with distilled water.
Repeat the first step with the solution pH = 4.01.
The pH meter is ready to use, do not change the settings.

*Third step*

Return the glass electrode to its sheath filled with distilled water. It can be used to measure pH values between 1 and 11. Return the reference electrode to its sheath filled with saturated KCl solution.

#### *pH measurement*

Perform a first measurement of the water sample.
Shake the water sample for 20 s to remove dissolved gases then measure the pH again.
Do several measurements and calculate the mean.

## 2B. Colorimetric measurement of ionic concentration

### *Aim*

This protocol tests the concentrations of chloride, copper, potassium and iron ions in three water samples using a kit for colorimetric determination of ion concentration.

### *Materials*

$Cl^-$ ions

- a container,
- a solution of $Cl^{-1}$,
- a solution of $Cl^{-2}$,
- a colour key for comparison.

$Cu^{2+}$ ions

- a cuvette,
- a solution of $Cu^{-1}$,
- a solution of $Cu^{-2}$,
- a colour key for comparison.

$K^+$ ions

- a haemolysis tube,
- a solution of $K^+$ ions,
- test strips for the assay,
- a colour key for comparison.

$Fe^{2+}$ ions and $Fe^{3+}$ ions

- a cuvette for the assay,
- a solution of $Fe^{-1}$,
- white powder of $Fe^{-2}$,
- a spoon to measure the quantity of powder,
- a solution of $Fe^{-3}$,
- a solution of $Fe^{-4}$,
- a colour key for comparison.

### *Protocols*

*Assay of $Cl^-$ ions:* kit Quantofix® (Macherey-Nagel)
Wash the container 3 times with distilled water.
Wash the container 3 times with the water sample to analyse.
Fill the container with the water sample to the 5 ml mark.
Add 3 drops of solution $Cl^{-1}$.
Add 4 drops of solution $Cl^{-2}$.

in this medium to give it a semi-solid consistency. This prevents the movement of inoculated organisms. Fluid thioglycollate broth has something for every microbe. Strict aerobes will grow only at the top of the tube, strict anaerobes only at the bottom, facultative and aerotolerant anaerobes throughout the tube, and microaerophiles somewhat below the surface.

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*Second step*

Remove electrodes and rinse with distilled water.
Repeat the first step with the solution pH = 4.01.
The pH meter is ready to use, do not change the settings.

*Third step*

Return the glass electrode to its sheath filled with distilled water. It can be used to measure pH values between 1 and 11. Return the reference electrode to its sheath filled with saturated KCl solution.

#### *pH measurement*

Perform a first measurement of the water sample.
Shake the water sample for 20 s to remove dissolved gases then measure the pH again.
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- a cuvette,
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- a solution of $Cu^{-2}$,
- a colour key for comparison.

$K^+$ ions

- a haemolysis tube,
- a solution of $K^+$ ions,
- test strips for the assay,
- a colour key for comparison.

$Fe^{2+}$ ions and $Fe^{3+}$ ions

- a cuvette for the assay,
- a solution of $Fe^{-1}$,
- white powder of $Fe^{-2}$,
- a spoon to measure the quantity of powder,
- a solution of $Fe^{-3}$,
- a solution of $Fe^{-4}$,
- a colour key for comparison.

#### *Protocols*

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Wash the container 3 times with distilled water.
Wash the container 3 times with the water sample to analyse.
Fill the container with the water sample to the 5 ml mark.
Add 3 drops of solution $Cl^{-1}$.
Add 4 drops of solution $Cl^{-2}$.
Mix and wait 30 s.
Place the container on the colour key.
Determine the concentration by comparing the colour of the solution to the colour on the key.

*Assay of $Cu^{2+}$ ions*: kit Visocolour® (Macherey-Nagel)
Rinse the container 3 times with distilleld water.
Rinse the container 3 times with the water sample to analyse.
Add 5 drops of the reagent $Cu^{-1}$ and mix.
Add 5 drops of the reagent $Cu^{-2}$ and mix.
Wait 5 min.
Hold the colour key and sample up to the light to determine the colour match and concentration.

Note: *If the colour is too weak, it means the concentration of copper is low. A white sheet of paper placed behind the sample can help to reveal the result.*

*Assay of $K^+$ ions*: kit Quantofix® (Macherey-Nagel)
Add 10 drops of reagent $K^{-1}$ into the tube.
Briefly dip the test strip into the sample to analyse.
Take out the strip and shake it to remove the liquid in excess.
Dip the strip into the liquid of the reaction tube.
Wait 1 min.
Take out the strip.
Match the colour on the strip to the colour key.
Presence of $K^+$ ions is evidenced by a yellow-orange colour

Note: *Do not touch the test zone on the strip with your fingers. Use forceps to hold it.*

*Assay Fe III*: kit Visocolour® (Macherey-Nagel)
Rinse the cuvette 3 times with the water sample to analyse.
Fill the cuvette to the 10ml mark.
Add 5 drops of $Fe^{-1}$ and mix.
Add one spoonful of $Fe^{-2}$ and mix.
Add 5 drops of $Fe^{-3}$ and mix.
Add 5 drops of $Fe^{-4}$ and mix.
Wait 5 min.
Hold the colour key and sample up to the light to determine the colour match and concentration.

Note: *If the colour is too weak, it means the concentration of iron is low. A white sheet of paper placed behind the sample can help to reveal the result.*

*Assay of Fe II*: kit Visocolour® (Macherey-Nagel)
Rinse the container three times with the water sample to analyse.
Fill the cuvette to the 10 ml mark.
Add 5 drops of reagent $Fe^{-1}$ and mix.
Add 5 drops of reagent $Fe^{-3}$ and mix.
Add 5 drops of reagent $Fe^{-4}$ and mix.
Wait 5 min.
Hold the colour key and sample up to the light to determine the colour match and concentration.

Note: *If the colour is too weak, it means the concentration of iron is low. A white sheet of paper placed behind the sample can help to reveal the result.*

### 2C. Photometric measurement of ion concentration

#### *Aim*

To determine the concentration of ammonium, nitrates and phosphates in three different water samples. We will use a photometer and a calibration curve.

#### *Protocols*

*Assay of ammonium ions for concentrations between 2.6 and 96.6 mg $l^{-1}$*: Kit Spectroquant® (Merk); solutions of ammonium chloride for the standard are prepared by the students from the powder (Sigma).
pH must be between 4 and 13.
Put 5 ml of $NH_4^{-1}$ in a tube.
Add 0.20 ml to the sample you want to analyse.
Add a small spoonful of $NH_4^{-2}$.
Mix until everything is dissolved.

Incubate for 15 min then pour the solution into a 10 mm cuvette and measure its absorbance at 712 nm.

*Assay of ammonium ions for concentrations between 6 and 193 mg $l^{-1}$*: Kit Spectroquant® (Merk); solutions of ammonium chloride for the standard are prepared by the students from the powder (Sigma).
Repeat with 0.10 ml of the sample to analyse.

*Assay of nitrates $NO_3^-$*: Kit Spectroquant® (Merk)
In the presence of sulfuric acid, nitrate ions produce a red compound.
Pour a small spoonful of reagent $NO_3^{-1}$ into a tube.
Add 5 ml of reagent $NO_3^{-2}$ and mix until the reagent $NO_3^{-1}$ is entirely dissolved.
Add 1.5 ml of the sample to analyse. Being careful, let the sample slowly slide down the wall of the tilted tube.
Wear protection glasses: the mixture becomes burning hot.
Mix while holding the upper part of the tube.
Let it stand for 10 min, then pour the solution into a 10 mm cuvette and measure the absorbance at 525 nm.

*Assay of phosphates $PO_4$*: Kit Spectroquant® (Merk); solutions of sodium phosphate for the standard are prepared by the students from the powder (Sigma).
In the presence of sulphuric acid, phosphate ions produce a yellow–orange compound.
pH must be between 0 and 10.
Pour 5 ml of the sample to analyse into a tube.
Add 1.2 ml of reagent $PO_4$ to the tube and mix.
Pour the solution into a 10 mm cuvette and measure the absorbance at 410 nm.

# Appendix 3: Economic approach

## Aim

To create a questionnaire to evaluate how much a person is willing to pay (WTP) for the preservation of a local ecosystem, including analysis and improvement of waste water treatment. The ultimate goal is to identify an economic issue of importance in order to design a project for ecosystem protection.

## Protocol

There are three steps.

Design of the economic survey.

1. Interviews.
2. Economic analysis of the results.

Material: Paper, pens (with different colours), laptop with internet access and a printer.

### *The questionnaire*

Students assume that the biochemical treatment of the WWTP is not optimal and that is why the local ecosystem suffers from pollution. They decide to ask the population how much they are willing to pay for the protection of their local ecosystem. They thus present a WWTP project involving better purification of the waste water, and the cost estimation for this project. This survey must contain 10 questions (open or closed) and a payment table. Each group creates its own poll. This economics session lasts 3 h.

### *The interviews*

Students present the project to different people as follows.

'The region is willing to spend a large amount of money to improve the treatment of waste water that goes into rivers to reduce water pollution. This project will solve all of the problems linked to water filtration by WWTPs and thus largely decrease the dramatic consequences on river fish.' In order to obtain pertinent results students need to interview a large number of people. An interview lasts around 8–10 min per person. One student reads and explains the questionnaire and one records the responses.

### *Results, analysis, and economic advice*

Materials: Laptop with Excel and PowerPoint software and a printer.

Data analysis and creation of the economic advice takes 2 h. Each group of students analyses its data in order to explain the average of the WTP (WTP*a*). The WTP*a* can be correlated to several factors: age, profession or gender of the interviewed persons. Students build tables and pie charts from the equations:

$$\Delta E = E_{ref} + E_{glass} = K + 0.006pHx \quad (1)$$

$$WTP = f(old). \quad (2)$$

At the end, the teenagers are able to propose some economic advice, for example the level of awareness of a population for this water pollution, and its WTP for a regional project that will improve water purification by WWTPs.

# Appendix 4: Social approach

## Aim

There are two main objectives: to work with students on concepts and language, and to help them formulate ideas. The second goal consist of guiding them towards a 'creative' session: creation of slogans within the framework of a fictitious awareness campaign intended for the public about aquatic ecosystems affected by wastewater treatment.

## Metaplan protocol

Material: a whiteboard, coloured cards

The activity begins with a couple of open questions, carefully formulated (the opening question was: 'What are the words that cross your mind when I say "Ecosystem"?'). Tutors propose four keywords (or 'stimuli words'), in connection with the theme of the course (*pollution*; *waste water*; *ecosystem*; *sustainable development*). Then, tutors ask students to spontaneously react to each keyword and to say the words that immediately cross their mind. Students write one or two words on coloured cards (each keyword corresponds to a colour). The tutor is responsible for collecting, grouping and sticking the cards on a whiteboard under the corresponding keyword. The next step of the metaplan is the reorganisation of the board: tutors ask students to move the cards in order to form large thematic assemblages and to give a title to each one. The poster produced is photographed. This poster is both the result of the metaplan and a working basis for the next phase (creative phase).

ıe objective is to get students thinking about their consumption of water and the environmental equences. They list the products they use every day, h are likely to contain pollutants (i.e. detergents, ctants, nitrates, various pollutants that persist in the ronment) and will be released in the environment. ıtors ask them whether they think it is possible to ume differently and whether less harmful products They usually come to the conclusion that envi- ıent-friendly products exist under the 'eco-label' wing criteria defined by the European Union. this discussion regarding consumption, students ss the following questions: Is it possible to influ- ways of consumption and change them? Who is to act? Who has to act? How can everyone be aware of this problem of wastewater? At this it is helpful to explain to students some key epts such as 'public policies', 'environmental poli- kers', 'citizens' and 'stakeholders'. The discussion ıblic awareness of the problem of pollution in ic ecosystems also included a discussion about the ency of public policies. Tutors used examples as government campaigns for road safety. Tutors lso ask whether, over the long term, photos might influence behaviour more than words. This discussion usually leads to the question: which medium would be the most effective for delivering a message?

## Slogan production

Tutors tell students: 'You are a team of creative advertisers recruited to develop a public awareness campaign. You have to build a communication plan'. Tutors ask students to create short sentences (slogans) in order to inform the public about pollution by wastewater, with the help of their previous brainstorming session with stimuli keywords (see above).

To assess whether students understood the overall architecture of the sessions and gauge especially the usefulness of the metaplan technique, tutors interview students at the end. The goal is to evaluate whether students appreciated how much the metaplan and discussion of consumption were a prerequisite for creative work (as part of awareness). Generally, they come to understand the connection between these processes, and the logic behind their use in these situations.