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Scientific Symposium

Small solutions for big water-related problems: innovative microarrays and small sensors to cope with water quality and food security

Istituto Superiore di Sanità
Rome, 26-28 October 2014

ABSTRACT BOOK

Edited by S. Marcheggiani, R. Spurio, E. D'Ugo and L. Mancini

ISTITUTO SUPERIORE DI SANITÀ

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ABSTRACT BOOK

Edited by
Stefania Marcheggiani (a), Roberto Spurio (b),
Emilio D'Ugo (a) and Laura Mancini (a)

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There is a close relationship between the quality of aquatic ecosystems and human health. This relationship stems primarily from the direct or indirect consumption of water polluted by toxic chemicals and/or contaminated by pathogenic organisms. Monitoring water quality is therefore of paramount importance for safeguarding public health and efficient primary preventive measures are required to cope with the rapid spread of pathologies linked to the progressive deterioration of the environment. The Meeting agenda includes a cluster of different issues such as the microchip technology applied to water quality evaluation; rapid and efficient methods to monitor the presence of emerging and re-emerging pathogens in aquatic ecosystems; aquatic biosensors; toxins detection in freshwater; detection of endocrine disrupting chemicals in water and liquids such as milk and fruit juices; cyanophages and the influence of climate change on aquatic ecosystems.

Key words: Environment and health, Water quality, Climate change, Emerging and re-emerging pathogens, Toxin detection, Cyanophage aquatic biosensor, Endocrine chemicals, Microchip technology.

Istituto Superiore di Sanità
Simposio scientifico. Dispositivi miniaturizzati per affrontare grandi problemi legati all'acqua: microarray innovativi e piccoli sensori per determinare la qualità dell'acqua e la sicurezza del cibo. Istituto Superiore di Sanità. Roma, 26-28 ottobre 2014. Riassunti. A cura di Stefania Marcheggiani, Roberto Spurio, Emilio D'Ugo e Laura Mancini. 2014, x, 69 p. (ISTISAN Congressi 14/C6) (in English)

Vi è una stretta relazione tra la qualità degli ecosistemi acquatici e la salute umana. Questo deriva principalmente dal consumo diretto o indiretto di acque contaminate da sostanze chimiche tossiche e/o microrganismi patogeni. Il monitoraggio della qualità delle acque è quindi di fondamentale importanza per la tutela della salute pubblica ,sono necessarie misure di prevenzione primaria efficaci per far fronte alla rapida diffusione di patologie legate al progressivo deterioramento dell'ambiente. Il programma comprende un insieme di questioni diverse: come la tecnologia microchip applicato alla valutazione della qualità delle acque; metodi rapidi ed efficaci per monitorare la presenza di patogeni emergenti e riemergenti negli ecosistemi acquatici; i biosensori acquatici; la determinazione di tossine in acqua dolce; il rilevamento di interferenti endocrini in acqua e liquidi come latte e succhi di frutta; I cianofagi e l'influenza dei cambiamenti climatici sugli ecosistemi acquatici.

Parole chiave: Ambiente e salute, Qualità dell'acqua, Cambiamenti climatici, Patogeni emergenti e re-emergenti, Rilevamento di tossine, Cianofagi, Biosensori acquatici, Interferenti endocrini, Tecnologia del microchip.

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The Symposium is co-organized by two EC consortia, the µAQUA (Universal microarrays for the evaluation of fresh-water quality based on detection of pathogens and their toxins) and RADAR (Rationally Designed Aquatic Receptors). The responsibility for the logistic aspects of the Meeting lies with the Department of the Environment and Primary Prevention of the Italian National Institute for Health (IstitutoSuperiore di Sanità, ISS).

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Annamaria De Martino	Ministry of Health, Rome Italy
Davide Di Cave	Tor Vergata Univerity, Rome, Italy
Luca Lucentini	Istituto Superiore di Sanità, Rome, Italy
Reyhan Akçaalan	Unilist Istanbul Univerisity, Istanbul, Turkey
Maura Manganelli	Istituto Superiore di Sanità, Rome, Italy
Giorgio Pennazza	University Campus Bio-Medico, Rome, Italy
Stefano Morabito	Istituto Superiore di Sanità, Rome, Italy
Stefano Fabiani	National Institute of Agricultural Economics, Rome Italy

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PROGRAM

Sunday, October 26, 2014

15:00 Registration

17:00 Welcome cocktail

Monday, October 27, 2014

8:30 Registration

9:30 Opening ceremony and welcome addresses
L. Musmeci
Director Department of Enviroment and Primary Prevention,
Istituto Superiore di Sanità

Plenary session 1
MONITORING WATER QUALITY FOR SAFEGUARDING EUROPEAN HEALTH

Chairman: **R. Mattioli**

9:45 *Challenges and synergies implementing EU directives for water management and flood protection*
G. Pineschi,

10:15 *Taking action to protect health from climate change*
B. Menne

10:45 *Climate changes and drinking water supply chain*
R. Colagrossi

11:15 Break

11:30 *Scientific challenges - Past and Future - in the implementation of the eu water Framework directive*
S.J. Eisenreich, A. van Griensven

12:00 *Radar: an innovative platform for remote surveillance of chemical pollutants and toxins*
S. Follonier, L. Varani, P. Colpo, T. Lettieri, L. Calzolai

12:30 *Opportunities in H2020: the call "Climate Action, Environment, Resource Efficiency and Raw Materials" 2014/15*
M. De Angelis

13:00 Break

Plenary session 2

ADVANCED METHODS FOR DETECTING EMERGING AND RE-MERGING PATHOGENS AND CONTAMINANTS AND FOR WATER DETOXIFICATION

Chairman: **C.O. Gualerzi**

14:30 *Microchip technology applied to the assessment of water quality*
W. Weigel

14:55 C*riteria for a successful microarray hybridization for pathogen and toxins detection in Freshwater samples*
D. Guillebault, J. Baudart, L. Medlin

15:20 *Water remediation and detoxification by electrochemical methods*
J. Bostock

15:45 *Towards the molecular evaluation of water quality levels: technical problems, successes and perspectives in the detection of biomarker diatoms by microchip hybridization*
L. Cimarelli, A. Brandi, L. Brandi, K. Saurabh Singh, B. Chandra Dhar, C.O. Gualerzi, R. Spurio

16:10 *Portable detection methods for toxin analysis*
K. Campbell, S. McNamee, C. Elliott

16:35 *Freshwater toxins detection: comparison of analytical and microspheres-flow methods*
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Poster session

17:00 Poster viewing

Tuesday, October 28, 2014

Plenary session 2

ADVANCED METHODS FOR DETECTING EMERGING AND RE-EMERGING PATHOGENS AND CONTAMINANTS AND FOR WATER DETOXIFICATION

Chairman: **J. Brookman**

09:15 *Rational design of estrogen receptors for biosensor development*
L. Varani, V.E.V. Ferrero, M. Pedotti, A. Chiado, L.Simonelli, L. Calzolai, T. Lettieri

09:40 *Label-free biosensor platforms for detection of endocrine disruptor pollutants*
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10:05 *Development of the new portable detection platform and the incorporation of biosensor and replaceable cartridge design*
F. Kehl, N.S.R.U. Tscharner, G. Etlinger, S. Follonier, J. Vörös

10:25 Break

Plenary session 3

POLLUTION AND ECOSYSTEM SERVICE

Chairman: **F. Manes**

11:00 *Cyanophage and their potential for controlling the development of cyanobacterial blooms*
P.K. Hayes, S. Watkins, J.E.M. Watts

11:30 *Factors determining whether or not cyanobacterial blooms will develop in reservoirs and lakes*
A. Walsby

12:00 *The detection of microorganisms in surface water as form of primary prevention*
S. Marcheggiani, E. D'Ugo, R. Giuseppetti; I. Fioramonti, A.M. D'Angelo, E. Pierdominici, L. Mancini

12:30 *Two-year monitoring of* Escherichia coli*,* Clostridium perfringens *spores, Adenovirus,* Giardia lamblia *and* Cryptosporidium parvum *occurrence in three rivers used as resource for drinking water production*
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13:00 Break

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ENVIRONMET AND HEALTH

Chairman: **E. Alleva**

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A. De Martino

14.45 *Environmental free-living amoebae in Italy*
D. Di Cave, F. Berrilli, R. D'Alfonso, M.S. Santoro, R. Monno, M. Montalbano di Filippo

15:00 *Water savingand plural uses: water resources potential for climate change mitigation*
S. Fabiani, P. Nino

15:15 *A reconfigurable liquid sensors network enabled by biorobotic technologies*
D. Accoto, A. Sudano, A. Alessi, R. Goffredo, F.R. Parente, A. Zompanti, G. Pennazza, M. Santonico

15:30 *Abattoir effluent Drains into rivers can cause environmental occurrence of pathogenic* Escherichia coli *and their transfer to vegetables*
K.M. Lawan, M. Bello, J. Kabir, L. Grande, V. Michelacci, R. Tozzoli, A. Maugliani, A. Caprioli, S. Morabito

15:45 *Early warning systems as an effective tool for the application of water safety plans to drinking waters: a case study*
L. Lucentini, E. Ferretti, L. Achene, P. Abis, E. Veschetti

16:00 *Verticaldistribution of a toxiccyanobacterium (Planktothrixrubescens) in an oligomesotrophic Turkish Lake (Lake Sapanca)*
M. Albay, R. Akçaalan, L. Köker

16:15 *Risk-based approach to assess and manage ostreopsis ovatablooms along the Italian coasts*
E. Funari, M. Manganelli, E. Testai

16:30 Selected posters discussion

18:00 Poster Award Presentations and Conclusion

20:00 Social Dinner

Plenary session 1

Monitoring water quality for safeguarding European health

Chairman
Raffaele Mattioli

CHALLENGES AND SYNERGIES IMPLEMENTING EU DIRECTIVES FOR WATER MANAGEMENT AND FLOOD PROTECTION

Giorgio Pineschi
Tutela delle Risorse Idriche dall'Inquinamento, Ministero dell'Ambiente e della Tutela del Territorio e del Mare, Rome, Italy

When in year 2000 the EU Water Framework Directive was finally issued after a long negotiation, the objective of good status for all water bodies within 2015 appeared ambitious but far to come at the same time. Today, approaching the deadline, Water Framework Directive still represent a fundamental pillar for EU environmental strategy and an international widely recognized best practice for all water related policies, even if good status will be met only for a bit more the half of European waters. About 53% of EU water bodies will be at good status in 2015 according to the estimation made by the EU Commission and EEA. EU Water Framework Directive introduced an innovative approach to water quality management, based on a comprehensive knowledge of key physical, economic and ecological information at river basin or water body scale, asking for an effective coordination among different EU, National and regional policies, especially in relation to the need to harmonize WFD and "Flood" Directive. Nowadays the assessment of the outputs of the first river basin planning cycle revealed difficulties and gaps. River Basin Management Plans (RBMPs) assessment also identified conflicts between water policy and other policy objectives that need to be addressed. The need to assess ecological quality status for surface water bodies represent one of the major challenge in WFD implementation, especially in relation to WFD Annex V requirements. First and second Intercalibration exercises clearly showed the level of technical expertise and scientific knowledge required to apply an exhaustive approach to ecological classification for all Ecological Quality Elements (EQBs). Italy identified more than 9.300 water bodies, 8.614 surface water bodies (88% are river bodies) and 733 groundwater bodies. Monitoring should be implemented in each of them, making clear the magnitude of the challenge, also under an economic point of view. Economic issues related to WFD Article 9 implementation also represent an important challenge. A National Working Group delivered in July 2014 a draft Guidance Document to support competent Authorities in assessing Environmental and Resource Costs (ERC). The challenge of WFD and Flood Directive implementation should be carried out, today and tomorrow, concentrating human and financial resources on main and fundamental objectives, fully applying the strategic approach set in the 2012 Blueprint initiative. A Blueprint to Safeguard Europe's Water Resources: Communication from the Commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the Regions.

TAKING ACTION TO PROTECT HEALTH FROM CLIMATE CHANGE

Bettina Menne
WHO Regional Office for Europe, Bonn, Germany

The health of human populations is sensitive to shifts in weather patterns and other aspects of climate change. Extreme weather events, more frequent, powerful, increased the risk of death and illness: 70,000 persons died in excess during the heat-wave period in 2003. Rising sea levels and increasingly extreme weather events will destroy homes, medical facilities and other essential services. A quarter of Europe's population lives within 60km of the sea. Increasingly variable rainfall patterns are likely to affect the supply of fresh water. Local changes in temperature and rainfall have altered distribution of some water- and vector-borne illnesses and contributed to the emergence of invasive vector species in Europe. As of WHO estimates, between 2030 and 2050, climate change is expected to cause approximately 250,000 additional deaths per year. The direct damage costs to health (i.e. excluding costs in health-determining sectors such as agriculture and water and sanitation), is estimated to be between US$ 2-4billion/year by 2030. Deep greenhouse gas emission reductions are required: valuing and incentivizing health-promoting mitigation actions in key economic sectors should be the cornerstone of any strategy. For example, cleaner energy systems, and promoting the safe use of public transportation and active movement - such as cycling or walking as alternatives to using private vehicles - could reduce carbon emissions, and cut the burden of air pollution, which causes some 7 million deaths every year. But health can also lead by example: the estimated carbon footprint for Europe's healthcare sector is similar to the emissions of international aviation and maritime transport activities of the EU Member States. The most effective adaptation measures for health in the near-term are programs that focus on prevention, ensure basic public health services, reduce inequalities, provide clean water, air and food, increase capacity for disaster preparedness and response, alleviate poverty, and strengthen health systems. For this reason, a more intensified inclusion of climate change into public health planning and programs is required as well as the identification of common approaches and metrics for monitoring and evaluation. This requires that governments put health sector preparedness and resilience in the centre of political attention, support resource mobilization, build capacity, innovate and promote technology transfer.

CLIMATE CHANGES AND DRINKING WATER SUPPLY CHAIN

Rossella Colagrossi
Direzione Generale della Prevenzione, Ministero della Saulte, Rome Italy

Extreme weather events intensified by climate changes, including unpredictable precipitation patterns, droughts, sea level rise, temperature variability, have significant implications for the sustainability of the water sector, particularly concerning drinking water suppliers. Capabilities in planning, assessing and adapting to climate changes effects is a dramatic need the drinking water chain should approach to fulfill their health, social, environmental and economic missions. The basic principles of the "Water Safety Plans (WSP)" provide for the implementation of a comprehensive and integrated strategy to reduce the health risk in the supply chain of drinking water. Strategies for adaptation to potential future scenarios should be incorporated in WSPs, in close connection with the world of research and innovation, with the aim of reducing the criticality and vulnerability of water systems from the infrastructure and management point of view. For example, in the study of materials suitable for contact with the water to use in the systems of collection, treatment and distribution may be important to include also the evaluation of the characteristic of resiliency of a material.

SCIENTIFIC CHALLENGES - PAST AND FUTURE - IN THE IMPLEMENTATION OF THE EU WATER FRAMEWORK DIRECTIVE

Steven J. Eisenreich (a), Ann van Griensven (b)
(a) Department of Hydrology and Hydraulic Engineering, IUPWARE, Water Resources, Vrije Universiteit Brussel, VUB, Brussel, Belgium
(b) UNESCO-IHE, Institute for Water Education, Vrije Universiteit Brussel, VUB, Brussel, Belgium

Water policy and its implementation in the European Union (EU) is embedded in the legal text(s) and subsequent guidance documents of the Water Framework Directive (and Daughter Directives), the Drinking Water Directive (DWD) and the Marine Strategy Framework Directive (MSSD). This presentation will focus in the limited time available on some of the scientific challenges faced by the EU in the implementation of the WFD, how successfully these challenges have been addressed thus far, and some further knowledge/science gaps still existing as the WFD moves toward post-2015. The implementation of the WFD has been possible with an insightful governance structure - the Common Implementation Strategy (CIS) - guiding progress and in the preparation of River Basin Management Plans by the MS. The scientific questions underlying the WFD's implementation (e.g., good ecological status, reference conditions, biological quality elements, EQs of PS/PHS, monitoring programs, relationship of ecological status to eutrophication, impact of climate change, intercalibration of water types across the EU) have been supported by the EC DG RTD to the amount of approximately **€** 460M over FP5 and FP6, FP7 spending > **€** 200M, and spending in H2020 of approx. **€**120M a year (broadly supporting 'Water'), with comparable amounts from EU MS. New scientific questions face the community on PS/PHS monitoring (incl. bio-monitoring) and risk assessment, the emission and monitoring and risk/impact of pharmaceuticals in surface and ground waters as well as DW. The issue of chemical mixtures and EDCs in European waters requires focus not only on analytical measurements but application of new molecular approaches to establishing activity and biological impacts as well the assessment of environmental modeling of chemical activity - truly a great challenge. And finally, the EC and partners has developed a prototype IPCHeM, the Information Platform for Chemical Monitoring Data, which aims to support a more coordinated approach to collecting, storing and accessing monitoring data on chemicals and chemical mixtures, in humans and in the environment (i.e., all media incl. biota) at the EU level. IPCheM is a de-centralised system, combining chemical information on a variety of environmental media, consumer products, food, and ultimately human beings providing the basis for understanding combined exposure and hopefully the effects of chemicals mixtures. (http://ipchem.jrc.ec.europa.eu/).

RADAR: AN INNOVATIVE PLATFORM FOR REMOTE SURVEILLANCE OF CHEMICAL POLLUTANTS AND TOXINS

Stéphane Follonier (a), Luca Varani b), Pascal Colpo (c), Teresa Lettieri (d), Luigi Calzolai (c)
(a) Centre Suisse d'Electronique et de Microtechnique, Zürich, Switzerland
(b) Institute for Research in Biomedicine, Bellinzona, Switzerland
(c) European Commission, Directorate General, Joint Research Centre, Institute for Health and Consumer Protection, Ispra, Varese, Italy
(d) European Commission, Directorate General, Joint Research Centre, Institute for Environment and Sustainability, Ispra, Varese, Italy

RADAR stands for Rationally Designed Aquatic Receptors integrated in label-free biosensor platforms for remote surveillance of chemical pollutants and toxins in complex matrices such as water and food (http://www.fp7-radar.eu/). The rationale behind the RADAR project is the need for a versatile biosensor platform for on-site measurement and continuous monitoring of pollutants and toxicants. The large number of different chemicals that have to be measured for their potential ecological toxicity (for example, as required by the REACH legislation) puts a huge pressure and at the same time, creates opportunities for the development of analytical tools, and the RADAR biosensor platforms is providing such novel tools. An efficient biosensor platform should be versatile to enable the specific detection of different chemicals (or chemical classes) with only minor modifications between different biosensors. An ideal platform would be able to simultaneously detect different chemicals in a mixture. RADAR has been focused on the rationally design of receptors which provide sensitive and specific biological recognition elements. The recombinant proteins that have been producedallow both an unparalleled control on the quality of the biological recognition elements and the introduction of “tags”for the simple and efficient manipulation of the sensing element. Several key features have been identified for the progress of environmental monitoring: the platform should provide easy, rapid, on-site measurements in the field; the sensors should be autonomous through the integration of wireless-network technologies. Another important key element is the miniaturisation of devices towards portable instruments, which is achievable due to advances in microfluidics and microelectronics, which reduce energy requirements and sample volumes.

OPPORTUNITIES IN H2020: THE CALL "CLIMATE ACTION, ENVIRONMENT, RESOURCE EFFICIENCY AND RAW MATERIALS" 2014/15

Miriam de Angelis
Agency for the Promotion of European Research, APRE, Rome, Italy

Horizon 2020 (H2020) is the biggest EU Research and Innovation programme ever with nearly €80 billion of funding available over 7 years (2014 to 2020), aiming at fostering breakthroughs, discoveries and world-firsts by taking great ideas from the lab to the market. H2020 is the financial instrument implementing the Innovation Union, a Europe 2020 flagship initiative aimed at securing Europe's global competitiveness. Seen as a means to drive economic growth and create jobs, Horizon 2020 has the political backing of Europe's leaders and the Members of the European Parliament. They agreed that research is an investment in our future and so put it at the heart of the EU's blueprint for smart, sustainable and inclusive growth and jobs. By coupling research and innovation, H2020 is helping to achieve such objectives with its emphasis on excellent science, industrial leadership and tackling societal challenges. The goal is to ensure Europe produces world-class science, removes barriers to innovation and makes it easier for the public and private sectors to work together in delivering innovation. Horizon 2020 is open to everyone, with a simple structure that reduces red tape and time so participants can focus on what is really important. This approach makes sure new projects get off the ground quickly - and achieve results faster. In the H2020 call 2014/15 on Climate Action, Environment, Resource Efficiency and Raw Materials'funds research and innovation with the following specific objectives:

- to achieve a resource - and water - efficient and climate change resilient economy and society;
- the protection and sustainable management of natural resources and ecosystems;
- a sustainable supply and use of raw materials, in order to meet the needs of a growing global population within the sustainable limits of the planet's natural resources and eco-systems.

Plenary Session 2

Advanced methods for detecting emerging and re-emerging pathogens and contaminants and water detoxification

Chairman
Claudio Orlando Gualerzi, Jayne Brookman

BIOFUNCTIONALIZATION AS KEY STEP IN MICROARRAY BASED APPLICATIONS

Wilfried Weigel
Scienion AG, Berlin, Germany

Array based tests have evolved into powerful tools for high-throughput analysis of a variety of classes of substrates as DNA, proteins, peptides, glycans, the detection of small molecule and screening of polymer properties. Especially DNA array technology faces broad application in environmental analysis and diagnostics. A key step in array based applications is the biofunctionalization that consists of chemical functionalization of the supports to introduce reactive moieties, deposition of the probes and the subsequent immobilization reaction. The main challenges in the immobilization of biomolecules are a high efficiency of the coupling step, the maintenance of bioactivity of the biomolecules and their accessibility in the subsequent incubation with a target molecule. Requirements on sample deposition are different for microarrays where a high number of spots are printed in a certain area in comparison to the loading of biosensors or lab-on-a-chip devices with often only one sample that has to be spotted at a predefined position on a support of a complex geometry. The coupling chemistry is well described in liquid phase, however, it is often neglected that miniaturization of assays using arrays from printing of drops with sub-nL volumes leads to a semi-dry state within seconds and the immobilization efficiency is determined by the local environment of the biomolecules that depends again from the design of the surface. The talk will focus on new technologies probe deposition techniques and strategies of probe immobilization to improve sensitivity, reproducibility and to minimize material consumption of array based tests in analytics and diagnostics.

CRITERIA FOR A SUCCESSFUL MICROARRAY HYBRIDIZATION FOR PATHOGENS AND TOXINS DETECTION IN FRESHWATER SAMPLES

Delphine Guillebault (a,b,c), Julia Baudart (a,b), Linda Medlin (a,b,c)
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Freshwater and drinking water quality assessment involves the specific and rapid detection of viable fecal bacteria indicators as well as toxic or pathogenic microorganisms in collected samples. Since the presence of these bacteria is often the main cause of noncompliance and subsequent boiling events, sensitive tools for detection of bacterial contaminants in distributed water is a major goal for utilities. Currently, water quality assessment requires time-consuming classical culture-based methods involving sample membrane filtration, incubation, and biochemical confirmation tests. However, depending on environmental pressures (starvation, oxidative stress, etc.), a variable proportion of bacteria can be disseminated in water in a viable but non-cultivable state. Development of efficient and rapid tests to monitor the microbial content in waters represents an essential health care strategy for control and prevention of diseases caused by waterborne pathogens. Molecular tools have greatly enhanced our ability to identify species, to determine their cellular activities or the expression of genes regulating key cellular processes and to estimate gene flow and species distribution in time and space. One of the μAQUA FP7 project goals was the design and development of a universal microarray for the detection of well-known and emerging pathogens and cyanotoxins in freshwater. A method was developed to amplify directly the intensity of the hybridization signal of the toxin genes directly on the array. The newly developed microarrays were tested with a large panel of samples collected from 12 European countries and spanning various environmental water bodies. Preliminary results from three French sites near Banyuls-sur-Mer including freshwater, brackish water and seawater are presented. The microarrays reduce energy requirements for water treatment, improve treatment performance and allow rapid management response under uncertain future climates.

TOWARDS THE MOLECULAR EVALUATION OF WATER QUALITY LEVELS: TECHNICAL PROBLEMS, SUCCESSES AND PERSPECTIVES IN THE DETECTION OF BIOMARKER DIATOMS BY MICROCHIP HYBRIDIZATION

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The advent of molecular techniques represents an important progress toward the detection and identification of microorganisms present in complex environmental samples, compared to traditional approaches based on the analysis of morphological features. In the past decade, molecular probe-based techniques have proved successful in improving both the efficiency and accuracy of the detection and identification of microorganisms. The aim of the present study was the design and development of a collection of oligonucleotide probes suitable for the identification of diatoms in microarray experiments. Diatoms are present in almost all aquatic and terrestrial environments and they are used as biological indicators to monitor the ecological status of water bodies. We focused our attention on a panel of 10 freshwater diatom species characteristic of five water quality levels. In addition, we developed molecular probes capable of identifying the marine diatom *Amphora coffeaeformis,* a potential producer of domoic acid, an harmful toxin responsible for human and animal deaths. Considerable effort has been devoted to the search for genetic markers that could provide the differences required for the design of species-specific oligonucleotides. In addition to the commonly used ribosomal RNA gene, we investigated the genetic differences present in the highly conserved genes coding for elongation factor eEF1A and the diatom-specific silicic acid transporter SIT. An alternative approach used in this study was directed to the use of genetic information deriving from a new technique, termed DIA-Tagging. In this case, rather than comparing conserved marker genes, we extracted and cloned random DNA fragments from each diatom genome. PCR-based DNA amplification was used initially to evaluate the accuracy of design strategy and the degree of specificity of the result and probes. Those oligonucleotide primers that proved positive in the preliminary assays were transfer reddirectly to the microarray format, or usedfor the design of oligonucleotide probes. Overall, at the end of design, test, refinement, cross-hybridization analysis and screening procedures of the microchip hybridization results, we obtained 12 oligo nucleotide probes based on SSU rDNA, 26 probes based on DIA-Tags and 12 and 20 probe stargeting eEF1a and SIT genes, respectively. These probes could be routinely used to monitor water quality in association with the standard EPI-D index available for the geographic area of interest.

PORTABLE DETECTION METHODS FOR TOXIN ANALYSIS

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The state-of-the-art in natural marine biotoxin analysis in seafood is now quite diverse with progress in moving away from the antiquated mouse bioassays. The future for bioanalytical methods requires cost-effective novel approaches to compete with the highly skilled multi-analyte detection now offered by laboratory based analytical approaches such as mass spectrometry. However, to realize demands for improved food security there is an increasing requirement for rapid portable tests for remote on site end product testing for managerial decisions from environment to farm to fork. To advance even further the state of the art it is no longer sufficient that rapid tests detect only one toxin or the structurally similar group. Advancements are required whereby biosensors should detect diverse groups of phycotoxins, mycotoxins or plant alkaloids as a single test. The difficulties of this approach with microarray platforms arise with regulatory limits and assay design.For marine and cyanobacterial toxins antibody based novel sensor methods offer model solutions but the greatest difficulty still arises with the detection of all analogues within a toxic group to meet regulatory demands. For natural toxins the design and application of broad specificity antibodies on multiplex and microarray platforms using a single combinational sample preparation offers this opportunity. High quality broad specificity antibodies to the toxin targets (microcystins; cylindrospermopsin, saxitoxin, domoic acid, okadaic acid, brevetoxin) were produced and fully characterised with regards sensitivity and cross reactivity. A planar waveguide immunoassay platform for the multiplex detection of the three key regulated groups of marine toxins and two cyanobacterial toxins also indicated to be an emerging issue in seafood has been developed. Toxin-protein conjugates were spotted onto sensor slides and molecular interactions between antibodies and conjugates were measured using secondary antibodies labelled with a fluorescent dye. The assays were optimised with regards sensitivity by using a chequer board of dilutions of the key reagents. The speed of the assay was optimised from 45 min, by studying the reaction kinetics, until a fully completed test could be performed within 15 minutes. The sensitivity (IC50) for each toxin group has been illustrated as 0.06, 0.42, 1.86, 1.40 and 0.19 ng/mL for saxitoxin, okadaic acid, domoic acid, microcystins and cylindrospermopsin in water samples. The assay demonstrates high suitability for toxin detection in seafood samples.

FRESHWATER TOXINS DETECTION: COMPARISON OF ANALYTICAL AND MICROSPHERES-FLOW METHODS

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Freshwater toxins are secondary toxic metabolites produced by cyanobacteria. These compounds can be divided in four groups: hepatotoxins, including Microcystins (MCs) and Nodularin (NOD), neurotoxins such Asanatoxin (ATX) and Saxitoxin (STX) and the protein synthesis inhibitor Cylindrospermopsin (CYN). To have rapid and accurate methods to detect these toxins in continental waters is important to avoid human and animal intoxications. The aim of this work is to develop multi-detection methods to simultaneously check for the presence of MCs, ATX, STX and CYN in freshwater samples. In addition, Domoic Acid (DA) is included in the detection set because this toxin has been recently related with freshwater/brackish environments. In this context, an inhibition assay using antibodies (to detect MCs, DA, STX and CYN) and a protein (ATX detection) in a microsphere flow-fluorimetryLuminex system is described. The method is a quick and simple tool for the detection of the five freshwater-toxin groups and allows for the first time their simultaneous detection. In addition, two analytical methods, a Liquid Chromatography coupled to Mass Spectrometry detection (LC-MS) method to quantify MC-LR, CYN, DA and ATX, and a LC coupled to fluorescence detection to quantify STX have beenimproved. Comparable results are obtained with the inmuno-detection and with the analytical methods when checking cyanobacteria cultures. Fast and easy results reporting the presence of the five toxin groups are obtained with the Luminex system,and new analogues with similar affinity to the proteins can be predicted. Besides, the method can be used for semi-quantitative screening purposes when only presence or absence data are requested and the amount is not necessary. However, more precise data, in terms of amount of each analogue are obtained with the analytical methods. In addition new analogues of each group can be identified within these methods although standards of each one are necessary for a correct identification/quantification. Next, both the Luminex system and the LC-MS method were used to study the presence of freshwater toxins in samples obtained at several localizations in Ireland, France, Germany, Bulgaria, Italy and Turkey. After a two years field study, small amounts of several analogues of ATX and some MCs have been reported in the water collected, while CYN, DA and STX presence were not detected.

DEVELOPMENT OF AN INTEGRATED, LABEL-FREE, WAVEGUIDE GRATING BASED BIOSENSOR PLATFORM FOR ON-THE-SPOT MEASUREMENTS AND ONLINE MONITORING OF TOXINS AND POLLUTANTS IN FOOD PRODUCTION PROCESSES AND IN THE AQUATIC ENVIRONMENT

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Endocrine Disrupting Compounds (EDCs) pose a significant hazard to the environment and human health. These are chemicals that can mimic natural hormones or inhibit their action by interacting with the receptors of natural hormones, thus altering the normal function of the endocrine, immune or nervous systems. In aquatic environment, all organisms are continuously exposed to EDCs for several life generations since most of them are persistent pollutants. Aquatic organisms have proteins capable of binding EDCs. Such proteins are ideal candidates as biological recognition elements for biosensors, since they will bind any EDC present in the analyzed sample.Here we present a fully integrated but versatile, label-free, evanescent field-enhanced waveguide grating based sensor system approach to monitor binding events of EDCs and toxins, using abovementioned aquatic biomolecules as signal transducers and introduce a biosensor platform for remote surveillance of EDCs, capable of monitoring the presence of toxins and pollutants in potable water as well as the aquatic environment.The label-free optical biosensor system ARGOS (Angle interrogated optical sensor) relies on a MEMS micro-mirror device to interrogate waveguide grating sensing regions on a dielectric optical transducer chip by scanning the angle of the incident coherent light, resulting in the determination of effective refractive-index changes on a chemically functionalized interface at a high acquisition rate. The tunable MEMS mirror allows interrogating a wide dynamic range and hence offers the flexibility to investigate at the point and wavelength of interest, an interesting feature for enhanced surface-to-bulk sensitivity ratio. The research leading to these results has received funding from the European Union's Seventh Framework Programme (FP7/2007-2013) under grant agreement n° FP7-KBBE-2010-4-RADAR 265721.

RATIONAL DESIGN OF ESTROGEN RECEPTORS FOR BIOSENSOR DEVELOPMENT

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Different chemicals present in surface, groundwater and marine waters around the world can interfere with the endocrine system. They can bind to Estrogen Receptors (ERs), thus affecting reproduction, embryonic development, and the nervous system. In particular, aquatic organisms are exposed for all their lifecycle and for several generations to Endocrine Disrupting Chemicals (EDCs) due to their persistence into environment, and accumulation into the food web as well as in drinking water. At present these chemicals are measured by using either analytical tools or toxicity assays, but they don't allow to detect and measure unknown compounds with EDC activity. The presentation will show results of an innovative approach of a 7-partner consortium (RADAR) which has developed a label-free biosensor using rationally designed biological recognition elements. Recombinant protein receptors have been designed to optimize two key properties: the ability to bind nanostructured surfaces and the binding of specific classes of compounds. Ligand Binding Domain (LBD) of the Estrogen Receptor alfa (ERα) has been selected for expression and purification. LBD is highly conserved amongst the human ER and the ERs from 100 different aquatic species (fish and amphibians). By combining sequence alignment and structural analysis of known ER-ligand complexes with computational analysis, it has been possible to predict single point variants with altered binding properties with respect to the wild type ER ligand binding domain . These predictions were experimentally confirmed by producing and characterizing the most relevant recombinant Era LBD variants. In particular, we were able to generate a single point ER mutant with a 6-fold increased binding affinity towards some EDCs (bisphenolic compounds and 17a-ethinylestradiol ligand). Due to the increased affinity, one of the ER variants, could be used as asbiorecognition element in an assay or biosensor resulting in higher sensitivity for the detection of class of compounds.

LABEL-FREE BIOSENSOR PLATFORMS FOR DETECTION OF ENDOCRINE DISRUPTOR POLLUTANTS

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There is increasing concern about many environmental contaminants that interfere with endogenous hormone systems, the so-called Endocrine Disruptors Compounds (EDCs). EDCs released into the environment can affect the endocrine, immune and nervous systems of animals causing many problems including reproductive disorders and cancers. It is therefore essential to develop robust methods to detect EDCs in food or water to reduce the risk of these pollutants on the human health and the environment. In this work an assay for the label-free detection of EDCs based on optical sensing platform based on surface plasmon resonance has been developed. Recombinant protein receptors, i.e. Ligand Binding Domain (LBD) of the Estrogen Receptor alpha (ERα), have been designed to bind (at high sensitivity and selectivity) EDCs in solution. Using an innovative approach based on the use of peptides that recognise specific conformation of the estrogen receptor-ligands complex, we achieved a strong signal enhancement for the detection platform. We will discuss the limit of detection for the β-estradiol and its comparison with other ligands. In addition, we will present the limit of detection of the estrogen receptor alpha and its mutants towards β-estradiol and other ligands.

Plenary session 3

Pollution and ecosystem services

Chairman
Fausto Manes

CYANOPHAGE AND THEIR POTENTIAL FOR CONTROLLING THE DEVELOPMENT OF CYANOBACTERIAL BLOOMS

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The problems associated with human health and ecosystem function due to the development of large populations of toxic cyanobacteria in freshwater systems, are well documented. From an understanding of the ecophysiology of these organisms and the physical-chemical features of the water bodies in which they occur, it is possible to model their population dynamics and, to some extent, attempt to manipulate conditions in the water column to prevent their accumulation. Once cyanobacterial populations have accumulated, however, the options for removing them from the water column are limited. Cyanophage, viruses that infect cyanobacteria, are ubiquitous and abundant in both marine and freshwaters. Cyanophages are known to play important roles in shaping the genetic structure of cyanobacterial populations, both through the selective lysis of particular genotypes or strains, and through their capacity to mediate horizontal gene transfer between cyanobacteria. It has been suggested that laboratory-prepared cyanophage innocula could be used as biological agents to effectively control cyanobacterial populations. In this presentation we describe the biological and practical problems associated with the implementation of such a control strategy. These problems include: the genetic diversity within the target cyanobacterial populations; the plasticity of cyanobacterial community structures; cyanophage host-specificity; host resistance to infection; the logistics of production and effective deployment of large cyanophage innocula and the impacts of environmental conditions on infection kinetics. Notwithstanding these problems, an improved understanding of the complex processes, ranging from phage attachment and infection, through to population dynamics after a phage infection, may facilitate the development of effective biological control strategies.

FACTORS DETERMINING WHETHER OR NOT CYANOBACTERIAL BLOOMS WILL DEVELOP IN RESERVOIRS AND LAKES

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Cyanobacteria are the only bacteria that possess both the green pigment chlorophyll and the blue pigment phycocyanin, which are essential for oxygenic photosynthesis. Molecular probes for the genes involved in the synthesis of these pigments are, of course, central to the identification of all cyanobacteria and not just those that inhabit lakes. But are there other genes particular to their life in lakes?Vaucher described the first cyanobacterium, *Oscillatoria*, in 1803, named for the oscillating movement of its filaments. Two centuries later more than 100 other genera had been described, and 80 species of *Oscillatoria* had been recorded. Taxonomical lumpers and splitters have had a heyday. Only a few of the many *Oscillatoria* spp. were found in the plankton of lakes; some of these have been designated as corresponding species of *Planktothrix*. What distinguishes them? Largely, the possession of gas vesicles, which provide the floating filaments with buoyancy. There are ten genes involved gas vesicle production, some highly conserved sequences and others with little sequence similarity. Species of *Microcystis* and other cyanobacteria forming large buoyant colonies occupy the surface waters of shallow unstable lakes where they float to the lake surface in calm periods, shading out other non-buoyant phytoplankton. Species of *Planktothrix* and other gas-vesicle containing cyanobacteria that remain as separate filaments, regulate their buoyancy and commandeer the metalimnion in deep stable lakes, where, with their red phycobiliproteins, they harvest the green light not absorbed by the green coloured phytoplankton in the water layers above them. The smallest cyanobacteria were not discovered until the 1970s. They pass through the finest plankton nets. With imperceptible sinking rates they needed no gas vesicles to stay afloat in the plankton. According to physical principles other benefits of their small size include the more efficient uptake of light and more efficient uptake of dissolved nutrients. They are, however, unable to move significantly relative to the water mass and their vertical distribution depends on movements of the water mass. Nevertheless, different species of these picoplanktonic cyanobacteria have acquired different phycobiliproteins some of which are adapted to the absorption of the wavelengths of light that penetrate to the bottom of the euphotic zone, at which there is zero net photosynthesis. The principle competition, then, between different cyanobacteria is for light, whose intensity and wavelength varies with depth. This is not, however, the sole competition. *Planktothrix rubescens* uses the last traces of light below the euphotic depth for the active uptake of amino and organic acids.

THE DETECTION OF MICROORGANISMS IN SURFACE WATER AS FORM OF PRIMARY PREVENTION

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Several studies have confirmed that water-related diseases not only remain a leading cause of morbidity and mortality worldwide, but that the spectrum of disease is expanding and the incidence of many water-related microbial diseases is increasing. The importance of water in the transmission of recognized pathogens is being constantly reassessed as advances in science, technology and epidemiology provide new tools for the evaluation and the analysis of the ecological status of water bodies. Most pathogens, bacteria, viruses, algae and toxins are small and tend to occur in low numbers, making them very difficult to measure directly. To better understand the link between environmental and human health the purpose of this study is to develop tools for the detection of bacteria and viruses in surface waters and improve the knowledge on 'emerging and re-emerging pathogens. The two sampling sites were chosen close to the urban area of Rome, one located upstream (Castel Giubileo) and another one located downstream (Mezzocammino). In each site fifty litres and five litres of raw water were sampled separately and later transported and stored at 4 degrees Celsius. Their analysis was performed within 24 hours according to the following procedure: fifty litres were concentrated into one litre of back flush solution and filtered through a membrane filter with different pore sizes following *µAQUA* protocols. Filters were pooled from each site and RNA and DNA extraction was performed and tested against microarrays using *µAQUA* protocols. Microbiological detection of *Camplylobacter, Clostidium, Salmonella* and *Staphylococcu*s was performed on aliquots of back flush solution and raw water. At the same time, the detection of enteric viruses, such as Human enteroviruses Human Hepatitis A and E, Norwalk viruses GI and GII and Human adenovirus F, was performed using qPCR , previous the extraction of nucleic acids. The results of this study showed that the level of pollution of the Tiber river increases considerably downstream as well as wide spread of emerging and re-emerging pathogens.. A significative concentration of *Salmonella, Clostridium, Campylobacter, Staphlococcus* and precence of *HEV, Noroviruses GI and GII and Adv 40 and 41* was detected in the Mezzocammino sample, while a smaller concentration of bacteria and only Adv 41 were recovered in Castel Giubileo. Microarrays testing is ongoing. The quality of the river's water decreases remarkably after its confluence with its main tributary, the Aniene river, in the city of Rome, probably as a result of the growing exploitation of its waters for agricultural, urban and industrial activities and effects of climate change (i.e floods).

TWO-YEAR MONITORING OF *ESCHERICHIA COLI*, *CLOSTRIDIUM PERFRINGENS* SPORES, ADENOVIRUS, *GIARDIA LAMBLIA* AND *CRYPTOSPORIDIUM PARVUM* OCCURRENCE IN THREE RIVERS USED AS RESOURCE FOR DRINKING WATER PRODUCTION

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Started in 2011, a two-year monitoring of physicochemical and microbiological parameters (*Escherichia coli*, *Clostridium perfringens* spores, adenovirus, *Cryptosporidium parvum* oocysts and *Giardia lamblia* cysts) has been conducted on three large rivers used as resource for drinking water production in France. Fifty-liter water samples were concentrated using hollow-fiber ultrafiltration and analyzed by molecular biology or laser-scanning cytometry. Correlations between measured parameters were statistically estimated, in order to attempt to identify point and non-point sources of pollution causing changes in microorganism concentrations. For instance, *Cryptosporidium* oocysts concentrations were significantly correlated with rainfall events, probably due to soil leaching and agricultural activities. Besides, *Escherichia coli* and adenovirus amounts were directly correlated, suggesting a common fecal pollution source. In addition, low temperatures appeared as favorable for *Clostridium perfringens* and adenovirus persistence in river water, potentially attributable to a seasonal effect. Health risk assessment will be as well calculated for each waterborne pathogen in the case of swimmer exposure during recreational activities.

Plenary session 4

Environmet and health

Chairman
Enrico Alleva

EARLY WARNING SYSTEMS FOR HEATWAVES EVENTS AND PREVENTION OF HEAT HEALTH EFFECTS

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In 2004, a small group of national experts, coordinated by the Operative Director of the Health Prevention, was committed by the Ministry of Health to realized guidelines about Surveillance and Action Plans with the aim of preventing and facing health effects from anomalous heatwaves. Italy was one of the first countries in Europe to activate a national prevention plan, called *Heat Health Prevention National Operative* Plan, which was developed in cooperation with the Ministry of Health, the Center for Disease Prevention and Control (CCM) and the National Department of Civil Protection (DPC), with the technical support of the Department of Epidemiology of the Regional Health Service. The prevention plan has five main objectives, as the spread of city-specific forecasting systems about heat effects; the setting of local response plan referred to specific categories at risk, like the elderly (the vulnerable person registry); the identification of reference centers responsible for surveillance and data and procedures spread in the early stage of a heat emergency; the communication campaign on the risks of heat every summer; the evaluation of systems aimed to obtaining more specific and timely information about daily mortality rate due to heatwaves. Currently, italian plan predicts the arrival of a heatwave with the advance of 72 hours and works according to a centralized control model; it supports the coordination between various monitoring levels (national, regional and local) and allows the implementation of surveillance and prevention activities between Ministry of Health, CCM, DPC, prefectures, medics, volunteers and the entire network of social and health services. The centralized coordination facilitates the wide spread of forecasting newsletters and daily update on the Ministry of Health's website. The coordinated activities encourages the sharing of tools and best-practices for the public health. Actually, the plan involves 34 cities with over 200,000 inhabitants, among which 27 cities with a specific forecast alarm system. The national toll-free call center "1500" is all summer long and supply advice and recommendations to prevent heatwaves damages. Every city has a system of close surveillance about daily mortality due to heatwaves. The mortality rate has significantly decreased in the last 10 years because of the ability of people to adapt to the increasing heat, but also thanks to the prevention plans. In conclusion, the national prevention plan for the health effects of heatwaves is an effective way to dealing with the risk of heatwaves and an example of centralized planning to face current and future extreme weather events.

ENVIRONMENTAL FREE-LIVING AMOEBAE IN ITALY

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The purpose of the present study was to identify free-living amoebae (FLA) from environmental sources, including well water, thermal baths and domestic tap water in Italy, and to characterize the isolates at molecular level to better understand the environmental distribution of species/genotypes associated with human disease, which is necessary for the development of appropriate control measures. In this study a total of 19 water samples collected in the Lazio region were examined for the presence of free-living amoebae. Moreover 35 water samples collected in the Puglia region were analyzed. The detection protocols included *in vitro* cultivation on NN-agar, and molecular investigations. Molecular characterization was obtained through DNA extraction according to the MICROKIT QIAamp DNA protocol (QIAGEN, Milan, Italy). For typing Acanthamoeba spp., the ASA.S1 region of Acanthamoeba 18S rRNA gene was amplified with specific primers JDP1 and JDP2. This region includes diagnostic region 3 (DF3). Detection of other FLA was performed via internal transcribed spacer PCR described by Tsvetkova, 2004. Assignment to species/genotypes was obtained by comparing the sequences with those available in GenBankTM through a phenetic analysis performed by MEGA version 5. Three out of 19 samples from Lazio (two thermal and one tap waters) resulted positive for Acanthamoeba by molecular detection; phenetic analysis allowed to assign the isolates to the genotype T4. Concerning Puglia, the phenetic reconstruction showed two monophyletic clusters corresponding to the genotypes T4, and T15. In particular, 9 Acanthamoeba isolates from public ornamental fountains and tap water were assigned to the genotype T4, while 4 isolates from artesian wells were identified as genotype T15. Concerning the detection of other FLA, 24/54 samples were found positive; in particular the molecular characterization of isolates allowed to assign 14 isolates to Hartmannella vermiformis. FLA infections are emerging diseases that gained, recently, much attention. These amoebae, known as free-living organisms, are cosmopolite and they have been isolated worldwide. Several species are implicated in human disease: *Acanthamoeba spp.*, *Hartmannella spp. Naegleria fowleri, Balamuthia mandrillaris* and *Sappinia diploidea*. The results obtained in the present study allowed to add new data about the occurrence of Acanthamoeba and other free living amoebae genotypes/species in environmental water samples in Italy by molecular techniques and genotyping. The results confirm that different water source in Italy may act as reservoirs of amoebic strains potentially pathogenic for humans.

WATER SAVINGAND PLURAL USES: WATER RESOURCES POTENTIAL FOR CLIMATE CHANGE MITIGATION

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Irrigation is the largest consumer of water resources with peaks between 60 and 80% of the total requirements in the regions of Southern Italy. Innovative methodologies are essential to obtain an accurate assessment of the volume of water used in agriculture to increase water availability for other uses. Lower volumes of water used in the fields also results in energy savings, and thus lower CO_2 emissions. In Europe, technological innovation led to the development and dissemination of monitoring systems of irrigation, particularly based on water balance and satellite observations, which entailed to the implementation of "Irrigation Advisory Services" (IAS). The development of these services in Italy today, represents a true excellence in sustainable water management in agriculture. Showing how tools based on the operational implementation of Earth Observation (EO) technologies can contribute to improve a more efficient water management at river basin level, the goal is to identify feasible and shared methodologies for sustainable management of water resources in agriculture, in line with the European Directive on Water n.60/2000. Furthermore, in the context of mitigation actions for Climate Change (CC), the move towards a low-carbon Europe with secure supply at affordable energy prices requires a profound transformation of the energy system, from production to demand, to strengthen the EU 2020 and 2030 energy targets. In this framework some appearance on the contribution of water resources concerns the potential in the field of micro and mini hydropower systems linked to existing channels in irrigated areas of irrigation consortia, where hydraulic storage, derivation and adduction schemes, cancontribute to better use of renewable energy sources, in a perspective of plural use of water resources. Today hydropower in Italy produces about 45 billion kWh/year and irrigation in consortia, with its potential 680 million kWh/year of mini hydropower plants, could cover about 1,5% of national hydropower production (approximatively 360.000 tCO_2 avoided). While for some consortia the use of hydropower can generate benefits in terms of reduced energy consumption, for many others the expected potential production from hydroelectric plants would cover the entire energy needs, even generating a surplus of production which results,due to the incentives system, in additional revenues.

A RECONFIGURABLE LIQUID SENSORS NETWORK ENABLED BY BIOROBOTIC TECHNOLOGIES

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The monitoring of large underwater environments is a considerable challenge for a number of reasons. One of the main challenges is posed by the need of constantly analyzing a large volume of water, without using a large number of network sensor nodes. To reduce the number of nodes the Authors propose a network of mobile chemical liquid sensors installed onboard of biomorphic robots. These robots, capable of 3D underwater locomotion, are able to monitor a volume of water larger than that possibly monitored by a fixed network of sensors. To achieve this goal, it is necessary to endow the robotic platform with a suitable dexterous swimming ability. Our platform exploits carangiform locomotion, implemented by actuating the three degrees of freedom of the tail. Dynamic diving is implemented by controlling the center of gravity position with respect to the center of buoyancy. Roll-pitch-yaw orientation is measured by three-axial magneto-inertial sensors, while depth is measured by a gauge pressure sensor. To avoid collisions, a custom obstacle avoidance system, based on ultrasound time of flight estimation, was developed. Furthermore the embedded electronics can integrate a localization system (GPS), which can be used in emersion phases. Underwater horizontal position can be estimated by sensors data fusion. The bio-robotic system is equipped with a grid of chemical liquid sensors. The sensing element consists of Flexible Screen Printed Electrodes (FSPEs) organized on the system surface in order to fit the geometric and fluid-dynamic requirements, but granting an optimal interaction with the measurand. These potentiometric sensors are controlled by an electronic interface devoted to input supply and output acquisition is designed to obtain a S/N ratio >1. The input consists of a triangular waveform between -1V and 1V with a frequency of 10 mHz. The registered output is a sequence of 100 current values. The final pattern is a multidimensional array composed of 100 virtual sensors, each referred to the current output response to a specific voltage input. These patterns, stored on board in a flash memory (SD card), can be analysed with multivariate data analysis techniques to provide classification, characterization and identification of complex chemical mixtures and/or solutions. Finally, these sensors are capable of detecting some pollutant which could be produced by local fauna, flora or artificial sources, sensing alteration from water reference sample.

ABATTOIR EFFLUENT DRAINS INTO RIVERS CAN CAUSE ENVIRONMENTAL OCCURRENCE OF PATHOGENIC *ESCHERICHIA COLI* AND THEIR TRANSFER TO VEGETABLES

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Escherichia coli is part of the microflora of the digestive tract of human and animals but certain strains evolved the capability to cause a wide range of disease. Pathogenic *E. coli* with a zoonotic origin, such as Shiga Toxin-producing *E. coli* (STEC), can be released with the wastes coming from the slaughterhouses in the environment where they can persist. We investigated the presence of pathogenic *E. coli* in specimens taken at an abattoir located in the Zaria region, Nigeria, and in water samples collected in the river Koreye, where the effluent from the abattoir spills in. Finally, we have evaluated the possibility that the pathogenic *E. coli* could be transferred from the abattoir to the crops irrigated with the water from the river. All the *E. coli* isolated from the samples were screened by PCR for the presence of genes associated with the know diarrheagenic *E. coli*. We could isolate three STEC strains positive for the presence of both the $stx_1$and stx_2 genes from the rectal content of two slaughtered animals and on cabbage sampled at a nearby farm where crops were irrigated with the water from the river Koreye. Additionally we have isolated one Enteroaggregative *E. coli* (EAggEC) from the abattoir effluent and two Subtilase-producing *E. coli* from the effluent and a sample of carrots grown at the same farm. Our results provide evidence that pathogenic *E. coli* can contaminate the environment as a result of the discharge of untreated abattoir effluent. In conclusion, the abattoirs and the surrounding environment, particularly in the absence of good practices and standard operating procedures for the slaughtering process, or proper treatment of wastewaters before their discharge in water bodies, might serve as a source for the environmentally mediated contamination of vegetables. Additionally, the low hygiene may favor the release and persistence of multiple pathogenic *E. coli* strains in the abattoir environment, including those belonging to human-borne pathogroups such as the EAggEC, making such a setting a unique favorable environment for bacteria to bacteria interaction and exchange of genetic material possibly leading to the emergence of new pathogenic strains with shuffled virulence features.

EARLY WARNING SYSTEMS AS AN EFFECTIVE TOOL FOR THE APPLICATION OF WATER SAFETY PLANS TO DRINKING WATERS: A CASE STUDY

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Although an adequate supply of safe drinking water is universally recognized as one of the major prerequisites for a healthy life, waterborne diseases are still a major cause of death in many parts of the world, particularly for children, and represent a significant economic constraint in many subsistence economies. This is caused by the fact that all water sources to be destined for human consumption may contain natural contaminants, particularly inorganic species that arise from surrounding geological strata and, to a varying extent, anthropogenic pollutants and microbiological contaminants. Waters delivered to consumers a recurrently judged safe for human consumption on the basis of national standards and international guidelines. The most important ones are WHO Guidelines for drinking-water quality (GDWQ), adopted as reference for regulation and standard setting in developing and developed countries worldwide. The GQWQ are revised on a regular basis and are supported by a range of detailed documents describing many of the aspects concerning the water safety. Since the third edition in 2004, they have supported the application of a comprehensive and systematic approach, called Water Safety Plans (WSP), which has proved to be the most effective strategy for maintaining a safe supply of drinking water to the consumers. One of the key components of this approach is the operational monitoring, which is a prerequisite to the identification of control measures in the drinking water systems. In this regards, the development of appropriate Early Warning Systems (EWS) for water quality surveillance may represent an effective tool for the implementation of WSP. An EWS can be defined as a system of data collection and analysis to monitor water characteristics to provide timely notice when an emergency threatens and thus to elicit an appropriate response. This definition emphasizes that EWSs are information systems with a specific objective, which is to provide information on occurring hazards that might evolve into harmful effects unless early response is undertaken. Therefore, the objective of an EWS is to monitor the first signs of emerging hazards in order to be able to trigger early and appropriate responses to these first signs and thus reduce or mitigate risk of harmful or adverse effects on the consumers. For an EWS it is fundamental to early detect a sudden abnormal change in any chemical, physico-chemical or biological variable before distributed water reaches the distribution system and the consumers. In the present research we have assessed the effectiveness of different EWS recently implemented at different steps of the drinking water supply chain within a wide water supply system located in Apulia (South of Italy).

VERTICAL DISTRIBUTION OF A TOXIC CYANOBACTERIUM (*PLANKTOTHRIX RUBESCENS*) IN AN OLIGOMESOTROPHIC TURKISH LAKE (LAKE SAPANCA)

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Sapanca Lake is a oligomesotrophic inland water body, with a surface area of 46.8 km2, a maximum depth of 53 m and a watershed area of approximately 300 km^2. The lake has been pristine for most of the 20th century. Last decades intensifying land use, increasing pollution in the streams draining to the lake and excess water withdrawals affected the lakes water quality and ecology adversely so that symptoms of eutrophication became noticeable. An extraordinary bloom of *Planktothrix rubescens*, which can produce Microcystins (MCs), was observed in early 2007 in the Sapanca lake with 76 µg/Lmicrocystin LR equiv. Due to a remarkable restoration program, the lake has been recovering after a remark able decrease in Total Phosphorus (TP), Nitrite + Nitrate (NO_2+NO_3), chlorophyll-a and microcystin-LR concentrations have decreased from 35-40 to15-20 µg/L; 300-330 to 100-120 µg/L,15-20 to 3-10 µg/Land 70-76 to 3-7 µg/L microcystin LR equiv, respectively. However, cyobacterial toxins tends to increase from 2012 to up to now. During this period it was fluctuated between 8-18 µg/L microcystin LR equiv. The lake water is a source of drinking water for two near by cities, Sakarya and Izmit. Therefore, water quality, especially toxin concentrations should be monitored in regular basis and the microarray could be a useful tool for monitoring efforts of the lake water.

RISK-BASED APPROACH TO PREVENT EFFECTS ASSOCIATED TO EXPOSURE TO *OSTREOPSIS* CF. *OVATA*, A MARINE TOXIC BENTHIC DINOFLAGELLATE

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We proposed a risk-based approach to implement the 2007 Italian guidelines on dangerous exposure to the toxic *Ostreopsis* cf. *ovata* during bathing and recreational activities on beaches. There are still important knowledge gaps, particularly on the toxins produced (mainly ovatoxins group and palitoxins) and their distribution in different matrices (cells, water, aerosol, fisheries products, etc.), or on the toxicological profile of the toxins. Therefore, we based our approach on the few epidemiological and toxicological data available on palitoxin and ostreocin-D (analogue of palitoxin) toxicity, assuming a similar toxicity for the ovatoxins. We defined three scenarios of exposure: by ingestion, by contact and by inhalation and assessed a provisional threshold for the oral route, derived from the acute reference dose of EFSA for palitoxin. Data on toxin quota per cell allowed expressing this limit as number of cells of 100.000 cell/L, considering an involuntary ingestion of 100-200 mL of water. Thresholds for the cutaneous and inhalation exposure (30.000 cell/L) have been empirically derived from the comparison of *O*. cf. *ovata* density and data on intoxication events from the Italian and French coast. We used these scenarios to define three phases of attention relative to monitoring plans: routine, alert and emergency, suggesting the actions to take at any moment. Parallel to environmental monitoring, we developed a multi-step syndromic-surveillance system, aimed at collecting important epidemiological information and at limiting unnecessary accesses to the hospital through a screening action by local workers (lifeguards, local health units, pharmacists, etc.).

Poster section

FIRST MONITORING NETWORK OF ALPINE SPRINGS IN NW ITALY FOR THE EVALUATION OF BIODIVERSITY AND THE PROTECTION OF AQUATIC RESOURCES

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Alpine springs are at the same time sentinels of global change and a potential water resource. With the aim of monitoring these environments, ARPA Piemonte has individuated 6 high altitude springs situated in NW Italy, in which the diatom benthic communities, although irregularly had already been monitored (2001-2014). The springs are situated at altitudes between 1,800 and 2,500 m a.s.l. in the upper part of the Pesio, Gesso, Maira, Germanasca, Sesia and Formazza valleys. Starting from 2014 each spring will be monitored yearly, including the following aspects:

- physical and chemical water variables;
- geological variables related to permafrost;
- benthic diatom communities;
- aquatic macrophyte communities (including the components macroalgae, bryophytes and superior plants);
- zoobenthic communities (separate sampling of meio- and macro-benthos);
- hygrophilous vegetation under the direct influence of the springs.

Detailed survey of the physical habitat of the outflowing brook in the stretch immediately downstream of the spring according to standard procedures. In the springs the water velocity wull be measured and thermometers linked to data-loggers will be progressively installed. The data derived from the spring monitoring will be elaborated and compared with those available from the Agency's meteorological-climatic network, considering the most representative stations for each spring. It will also be possible to use mobile meteorological stations. What is described above is the basic nucleus of a long-term monitoring network of high-altitude alpine springs; an interaction will be possible with other national or international subjects, necessary for methodological standardization. Springs are in fact environments connecting ground and surface waters, scarsely considered by existing legislation and generally not included in national freshwater monitoring networks. It is planned to introduce monitoring of part of the springs with the technique of microarrays, in collaboration with other organizations. The wide range of monitored aspects and the the optimal integration of the information render this network unique among the experiences of alpine spring monitoring, because it brings together several approaches linked to the protection of alpine spring biodiversity and aquatic resources. The role of diatoms is fundamental because they are a stable, well-known component of these systems, rich in information on the integrity and extreme environmental conditions of high-altitude

springs. The described monitoring network will prove useful for obtaining important information for managing and protecting these mountain environments that are sources of water and biodiversity for the surrounding territory.

DETECTION AND QUANTIFICATION OF RESIDUES AND METABOLITES OF PHARMACEUTICAL COMPOUNDS IN ENVIRONMENTAL COMPARTMENTS, FOOD COMMODITIES AND WORKPLACES. A REVIEW

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The toxicological assessment of residues of pharmaceuticals and of their degradation products, mainly antibiotics, antitumor drugs, antidepressants and hormones, in the environmental compartments, workplaces and foodstuff and feedstuff have become a challenging task worldwide. Their presence poses nowadays a serious threat to human health and to environmental integrity which is still far from being fully understood. The growing concern about pharmaceuticals as contaminants stems from their physical and chemical behavior resembling that of other persistent xenobiotics causing potentially adverse effects. The striking advancement made by medicine has had undeniable benefits for the society as a whole, primarily because of the widespread availability of pharmaceuticals in the medical practice to effectively treat most pathologies. On the other hand, the toll paid is basically due to the still limited understanding of the effects of the discharge of such substances into the environment independent of whether this is the consequence of their proper use or of fortuitous contamination processes. This challenge is ever more attracting the attention of the international scientific community, the decision makers and the layman and concern has been expressed over the deleterious consequences of the discharge of pharmaceuticals, often as unused or expired products and as undue release in workplaces. The widespread occurrence of pharmaceuticals raises a number of questions out of which of prime importance is the reliable quantification of their concentrations in the various media to evaluate whether they can pose an appreciable risk for exposed biota and human beings. A particular attention is posed on determination of antibiotics in the environment and food products, still a major challenge requiring further substantial efforts in the development of quick analytical methodologies as the innovative approach based on selective and sensitive biosensors based on synthetic RNA or DNA single-stranded oligonucleotide sequences (aptamers). This review deals in particular with the most promising analytical and bioanalytical approaches developed so far to quantify such contaminants in environmental media, food products and workplaces. More, an attempt is also made to outline possible future trends also in the view of regulatory requirements.

DIATOM_EQR_IT: SOFTWARE FOR CALCULATING INDEX ICMI USING DETECTION OF DIATOMS

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The software is designed for Windows 7 using the Visual Studio development environment, with Framework 4.0 in C # language. The application was designed to be used exclusively by users via the website, and uses simple Excel file for input and output of managed data and the responses processed by the software. Regarding the storage and back-up of data processed, the software manages its website area, on its hard disk, dedicated to storing data files posted by users and its output files containing the results of the processing. These Excel files (on site server) are uniquely identified by the code-user and other parameters entered when accessing the website. To use the software, at the beginning, the user must download a pre-formatted Excel file from the site, called "Input_Dati_Template.xls", to be used later to load the data of the various samples to be processed. This file contains an updated list of species catalogued in the system, in which there are code Taxa and their nomenclature (for each species), and can be saved on the local disk to be used at other times, giving to it a different name depending on the associated sample. The entire list of species available within the input Excel file simplify the search for the desired species directly using the search functionality of Excel, working off-line, without connecting to the website. The input file data involves the insert of river macrotype and the list of abundance of the various diatoms detected. At the end of data entry, the user uploads the Excel file to the website for processing. This step of processing works on the last input-data file sent by the user: the software reads the data in the file and executes the control test for their validation. In the event of one or more errors detected, the software sends a copy of the last Excel input-file, sent by the user, containing the response error messages written next to the original data. If the data sent is correct, the software creates a new Excel output file containing the original sheets, with the data entered by the user, and a new sheet with the calculation results. This response file is stored in the website storage and immediately sent to the user, that can download it into own local computer.

METHODOLOGICAL ASPECTS IN THE TREATMENT OF ENVIRONMENTAL SAMPLES (WATER AND SEDIMENT) FOR ECOTOXICOLOGICAL ANALYSIS

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The eco-toxicological analysis are an important aspect in the multidisciplinary approach to environmental assessment. Thus, the samples processing plays a key role in the environmental analysis. In this study, the environmental sampling was carried out in an area under strong natural and anthropic pressures; the samples (sediment and water) were treated using the technique of filtration and concentration, as suggest within the european project μAQUA; at the same time, tests were carried out using environmental samples without treatment (water). *Vibrio fischeri* and *Daphnia magna* assays and the Micronucleus (MN) tests were performed to assess the environmental quality. The Microtox® bioassay testing system uses bioluminescent marine bacteria (*Vibrio fischeri*) to detect toxic substances in water. When exposed to a toxic substance, a change in luminescence is observed, probably due to the respiratory process of the bacteria. This change can be used to calculate the inhibition of *Vibrio fischeri* directly correlated to toxicity. *Daphnia magna* is a freshwater crustacean, whose sensitive offspring is used in the bioassay to monitor water quality. In normal conditions, the breeding is carried out by parthenogenesis, so that the population of daphnidi is composed of only young adult females of various ages with the same genetic makeup. Also for this reason, *Daphnia magna* is very often used in ecotoxicology, so that the genetic variability is greatly reduced although *in vivo* tests are performed. At the end of 24h and 48h toxicity tests, the total number of offspring (sorted by mortality of immobility) is assessed to determine the observed effect of the test substance. The Micronucleus (MN) assay is used to test the numerical or structural chromosomal damage induced by chemicals. In this study, it has been performed on a plant (Vicia Faba). If the cell division is disrupted, or the chromosomes are damaged by chemicals or radiation, pieces or entire chromosomes can be affected and the genetic material that is not incorporated into a new nucleus may form its own "micronucleus" which is clearly visible with a microscope. The analysis shows that the concentration of environmental samples results in more effective method compared to the analysis of the filtrated samples (sediment) and without treatment samples (water). Furthermore, the concentration of samples allows to test larger quantities of water from the environment, especially by a river, adding information about the real state of the environment or about the potential biological risk.

DEVELOPMENT OF A VALIDATED MEASUREMENT PROCEDURE FOR POLYCYCLIC AROMATIC HYDROCARBONS (PAH) IN SUSPENDED PARTICULATE MATTER SAMPLES AT A CONCENTRATION LEVEL REQUIRED BY THE EU WATER FRAMEWORK DIRECTIVE (WFD)

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The control of water pollution is an important issue with a large impact on human health and environment. The European Water Framework Directive (WFD 2000/60/EC) establishes a framework for the Community action in the field of water policy. It provides a list of priority water pollutants considered hazardous to or via the aquatic environment. Environmental Quality Standards (EQS) have been defined for these substances. For reliable measurement of contaminants at EQS levels in waters analytical methods are required to be in accordance with the Quality Assurance/Quality Control QA/QC Directive 2009/90/EC. A serious lack of applicable validated analytical methods able to meet the QA/QC requirements for contaminants such as TBT, PBDE and some PAHs (Tributyltin, Polybrominated Dipheyl Ethers and Polyciclic Aromatic Hydrocarbons) in whole water has been stated. For this reason EURAMET an implementation structure of the European Metrology Research Programme (EMRP) approved a Joint Research Project (JRP ENV08 WFD- Traceable measurements for monitoring critical pollutants under the European Water Framework Directive WFD-2000/60/EC) funded by the European Union. The aim of the Project was to develop validated measurement procedure for TBT, PBDE and PAH in whole water sample at a concentration level required by the EU WFD and in compliance with the Quality Directive. ISPRA participated in the Task 3.2 of the Work Package 3 of the EMRP ENV08 WFD. This task had the object to define the most promising extraction method of 8 PAHs in SPM (Suspended Particulate Matter), up to 500 mg/L, comparing the pressurised liquid extraction (PLE) and Microwave Assisted Extraction (MAE). ISPRA defined and validated a measurement procedure, according to International Standards and Guidelines, based on the MAE technique. The final results are presented. The EMRP is jointly funded by the EMRP participating countries within EURAMET and the European Union.

MICROCOKIT PROJECT - MICROBIAL COMMUNITY-BASED SEQUENCING ANALYSIS LINKED TO ANTHROPOGENIC PRESSURES TO ADDRESS THE WATER QUALITY

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Microbial communities are the base of the food web pyramid, representing about 50% of the total biomass on Earth. They are responsible for the geochemical cycles and bio-removal of organic compounds, including xenobiotics, playing a key-role in ecosystem functioning and providing several ecosystem services. They are able to adapt promptly to environmental changes and the presence of a natural microbial community is a necessary prerequisite for an effective response to the various chemicals that can contaminate an ecosystem. However, the recovery from contamination is only possible if toxicity does not hamper microbial activity. The knowledge of natural remediation capacity of a microbial community allows to assess the contaminant availability to higher levels (including man) in the ecosystem food web. The MicroCokit Project a close collaboration of academic groups with pan-European governmental laboratories, leading private enterprise and coordinated by CNR-IRSA, has been conceived to: 1. investigate and identify aquatic complex stressor indicators based on microbial communities; 2. foster the transfer of knowledge among the partners with the final goal to bring to market faster, more sensitive and robust tools as bioindicators of water quality.

ALGAL TOXIN DETECTION TECHNOLOGIES

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The effects of nutrient pollution in surface waters may cause algaeto increase to potentially harmful levels. Algae deplete oxygen andcertain species of blue-green and red algae can even poison aquatic life and pose a threat to human, domestic animal or livestock health. This poster summarizes the algae and algal toxin detection technologies provided by Modern Water and distributed in Italy by Ecotox LDS. The rapid detectionmethods for algae and algal toxins include bothimmunoassay test kits and fluorometers:

1) immunoassay is an analytical method which uses an antibody as a reagent to quantify specific analytes. The technique incorporates the binding reaction of a target substance (antigen) with an antibody;
2) fluorometers work on the principle that different molecules absorb and emit light at specific wave lengths. Modern Water fluorometers use LED light source to excite the molecules and then measure the emittance at the desired wave length. The intensity of the emitted light provides the concentration of the target compound.

Modern Water enzyme based immunoassay test kits produce results in as little as 45 minutes and detect a variety of algal toxins: Cylindrospermopsin (0.1 to 2.0 microg/L), Microcystin (0.3 to 5.0 and 0.1 to 1.6 microg/L), Okadaic Acid (0.2 to 5.0 microg/L), Neo-Saxitoxin (0.03 to 1.0 microg/L), Nodularin (0.04 to 1.0 microg/L), Brevetoxin (0.1 to 2.5 microg/L), and Saxitoxin (0.02 to 0.32 microg/L). These immunoassay methods offer a cost-effective solution for high through put sample testing. A yield of up to 80 samples can be achieved per 96-well microtiterplate, depending on batch size. Modern Water range of fluorometers are able to instantly detect Chlorophyll a, and the pigments phycocyanin and phycoerethrin in herent in potentially harmful blue-green and red algae. The fluorometer, AlgaeChek Ultra, allows the early detection of the pigments in herent in potentially harmful red and blue-green algae. The ELISA plate and tube tests, EnviroGard, can detect trace concentrations of the specific toxins that are generated by the Harmful Algal Blooms (HABs). Algae fluorometers are rugged and portable for rapid field deployment. The single parameter AlgaeChek can be programmed to detect Chlorophyll a, phycocyanin or phycoerythrin. Additionally, the AlgaeChek Ultra combines capabilities to detect all three pigments with one instrument.

THE ACTIVITY ON EFFECT-BASED TOOLS IN THE CONTEXT OF THE WATER FRAMEWORK DIRECTIVE: FUTURE PERSPECTIVES

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In the context of the 2010-2012 mandate of the Common Implementation Strategy (CIS) of the Water Framework Directive (WFD) an activity on effect-based tools, chaired by Sweden and Italy, has been launched with the aim to support the link between the chemical and the ecological status classification and to improve the chemical monitoring programmes of the WFD. In march 2014, after the approval and endorsement of the strategic coordination group and the water director meeting of the WFD, a technical report on aquatic effect-based monitoring tools has been published. The content of the technical report includes a background on current use and regulatory relevance, sections on bioassays (*in vitro* and *in vivo*), biomarkers, ecological indicators (e.g. SPEAR), EDA (effect-directed analysis) and OMICS technologies. Furthermore a specific chapter is dedicated to the possible use in the context of the specific monitoring programmes of the WFD. The activity on effect-based tools ranked very high in the CIS Science-Policy Interface (SPI) elaborated on the basis of a wide activity that has involved several member states and stakeholders and a follow-up has been decided for the mandate 2013-2015 of the CIS-WFD. The main activities are currently related to a focus on the evaluation of the integrative screening of pollutants and a field trial on the application of promising bioanalytical methods for the monitoring of estrogenic compounds such as the pharmaceuticals (e.g. EE2 and E2); both mentioned substances are included in the watch-list of the Directive 2013/39/EU on the priority substances.

INVESTIGATION OF CAUSES RELATED TO THE INCREASE OF NON INDIGENOUS SPECIES ALONG SOUTHERN ITALIAN COAST

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Records of non indigenous aquatic species along the southern Italian coast have recently increased, some euryhalinealien species can be considered established,as they easily acclimated to this area. In the peninsular ionic area an excessive erosion has taken place which caused in some cases a total loss of sandy habitats within few years. This is the case of Metaponto, located along the southern ionic coast between the Basento and Bradanoriver mouths. Such erosion has been related to different causes: river dredging, the presence of dams, the exploitation of water springs and to climatic changes. In facts, during the past ten years numerous floods occurred which were quite devastating for the area. Furthermore, during the last ten years the atmospheric temperature has increased in comparison to the previous 40 years. The climatic and habitat changes have probably favored the introduction of the American crab (*Callinectessapidus*) in this area, where the several rivers, canals and river mouths represent an ideal environment for its growth reproduction and propagation.The blue crab is among the most dangerous alloctonous crustaceous for aquatic fauna, especially in the mugilidae area. The species shows a wide environmental tolerance, it is euryhaline, eurythermal and can tolerate oxygen values lower than 0,08 mg/l, this characteristic confer a high colonization ability to the species. The blue crab can prey on fish, mollusks and crustaceous, dead animals and macrophytes can also be part of its diet. It can exert a strong effect on the colonized habitats as its alimentary habits can induce great modifications on the environment. The species is also highly prolific as it can produce from 2-8 million eggs per spawn. There are evidences from recent studies that indigenous species are decreasing to the benefit of alien ones in some southern Italian basins. The scenario is quite alarming and confirm the threat posed by alien species, aggravated by anthropic factors and the evolution of climatic changes, which are still far from being completely understood.

A YEAST-BASED PROBE TO DETECT ACTIVE CONCENTRATIONS OF HERBICIDES WITHIN THE ITALIAN PROJECT "ALERT"

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The "ALERT" project (http://www.alert2015.it) funded by the Italian Ministry for Economic Development pivots around the transfer to field situations of the sensor/biosensor platform BEST, patented by ISS. Within ALERT's frame, this PhD research looks at the detection of heavy metals and water soluble herbicides with plant- and microorganisim-based biosensors namely yeast (*Saccharomyces cerevisiae)* coupled with respirametric measurement and algae (*Chlamydomonas reinhardtii)* together with fluorescence technology, in water and milk. A preliminary study was carried out with the yeast system on acute and chronic effects of diuron (herbicide inhibiting the photosystem II in the plants). The test investigated the possible self-detoxification capacity of yeast cells: aerobic respiration was taken as the toxicological end-point. Inhibition is shown by increased dissolved oxgyen (ppm) after the addition of herbicide which gives a positive or negative percentages of interference value, after exposure for different time to herbicide. According to Directive 89/778/EEC, the maximum concentration for each single chemical (including pesticides) in potable water is 0.1ug/L; using this information, three different concentrations were investigated, i.e., 1.5 ug/L (10^{-6}M, one magnitude order above the limit), 0.015ug/L (10^{-8}M, one magnitude order below the limit) and 0.0015 ug/L (10^{-10}M, two magnitude order below the limit). Cells were hydrated with MilliQ water 12 hours prior to the experiments without addition of nutrients, in order to prevent cell proliferation. The cells were then exposed to the test concentrations over two different treatment periods, 6 and 24 hours, for the observation of acute and cronical toxicity, respectively. Percentages of interference were calculated for 6 hour exposure (-3.27%, 10.52% and 9.78% for 10^{-6}M, 10^{-8}M and 10^{-10}M respectively) showing that diuron inhibited cells' respiration at lower concentrations, whereas the top concentration might elicit an initial increase of cellular respiration. Over the 24 hour exposure period, 5.85%, 7.88% and 6.70% were obtained at 10^{-6}M, 10^{-8}M and 10^{-10}M respectively; therefore all concentrations inhibited cell respiration, however the top concentration showed a lower effect. High dose 10^{-6}M with acute exposure shows a slight increase of respiratory activity which may indicate a qualitatively different effect associated with the induction of a cellular metabolism of diuron not been induced at lower doses. 10^{-6}M in

chronic exposure begins to do the same effect as lower dosesbecause metabolic pathway may be saturated. These experiments also show the biosensor is very sensitive: giving effect to 2 magnitude orders below the limit.

THE DIATOM COMMUNITIES OF THE WELLSPRING ENVIRONMENTS IN THE APENNINES MOUNTAINS OF ABRUZZO, IN THE CONTEXT OF CLIMATE CHANGES

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A new study is currently being carried on in order to analyze and classify, for the first time ever, the diatom communities living in the wellspring environments of the Apennines mountains in the Abruzzo region, and compare the data obtained with those previously collected in the area of the Aterno river. As a result, chemical and physical data are now available for all samples collected and analyzed, as well as all lists of the diatom species found and identified on the site: for the campaign conducted in May 2014, the exact number of diatoms for each species is also available, while for the campaign of July 2014 this information is still being processed and has not yet been reported. The sampling activity was conducted using different tools to collect the samples of the different diatoms species: a tooth brush, used to scrape samples of epilithic diatoms from pebbles, a box-cutter, used to collect samples of epiphytic diatoms from the macrophytes, and a Pasteur pipette, used to retrieve samples of epiphelic diatoms from silt. In total the research identified 26 species of diatoms from the pebbles, 30 species from macrophyte and 46 species from the silt: in the first group (epilithic diatoms) the predominant species were Achnanthidium minutissimum, Gomphonema tergestinum, Gomphonema angustum and Meridion circulare, followed by Navicula tripunctata, Navicula reichardtiana, Gomphonema micropus and Amphora inariensis; in the second group (epiphytic diatoms), the predominant species were the Achnanthidium minutissimum, Diploneis separanda, Navicula cryptotenella, Ulnaria biceps, Nitzschia palea and Planothidium rostratum; in the third group (epiphelic diatoms), the predominat species were Achnanthidium minutissimum and Meridion circulare, Gomphonema micropus and Gomphonema tergestinum. The researchers believe that it is possible to identify specific aspects relative to the climate and its changes, and that this will ultimately enable to deduce any existing correlation between the presence and/or abundance of particular diatom species and climate changes, using a network of remote automated monitoring devices which can easily collect and store a sufficient number of reliable and accurate data on the diatoms living in the nearby environment.

A MEDIATED AMPEROMETRIC SENSOR COUPLED TO AN HAEMOLYTIC-ENZYMATIC ASSAY FOR THE DETECTION OF PALYTOXIN IN MUSSELS

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Palytoxin (PlTX), produced by benthic dinoflagellates of the genus Ostreopsis, is one of the most potent marine toxins. In recent years, due to the climatic changes, *Ostreopsis spp* has been found worldwide, also in the Mediterranean Sea, so palytoxin is now regarded as an emerging toxin. In fact, occurrence of *Ostreopsis spp* may result in PlTX contamination of seafood. In order to protect the human health there is the need to develop rapid and sensitive detection methods of PlTX and its analogues (PlTXs). Several methods for PlTXs have been reported in the literature but their application to shellfish is very poor. We have developed a mediated amperometric sensor for PlTXs detection, based on the measurement of lactic dehydrogenase (LDH) released into the medium when sheep erythrocytes are lysed after a short incubation time with PlTXs. In this study we have assessed the applicability of this method for PlTXs detection in mussels. A simple and rapid extraction procedure has provided the best results in terms of the matrix interference on the palytoxin detection. This extraction procedure included: blending of 10 g of whole mussel tissue with 90 ml of PBS, centrifugation, filtration, shaking with chloroform (1:1 v/v) for 15 min followed by a short centrifugation, collection of the aqueous phase and, finally, a dilution 1:50 in PBS. The evaluation of the matrix effect was performed by spiking the mussel extract with different concentrations of PlTX and by comparing them with the same PlTX concentrations realized in PBS. This extraction procedure allowed a satisfactory removal of the matrix interference. Preliminary results about the efficiency of the extraction have been obtained on mussels experimentally contaminated with PlTX and a recovery of about 75% has been estimated. Further experiments are needed to confirm this recovery. Our method, rapid, specific, sensitive and cost-effective can be an usefull tool to detect PlTXs in mussels.

MOLECULAR DETECTION OF ENTERIC VIRUSES IN FRESH WATER FROM EUROPEAN COUNTRIES

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The transmission of water-borne pathogens occur in the majority of cases by fecal-oral route, by inhalation or by direct or indirect contact with contaminated water.Previous molecular studies have allowed the identification of viral particles of both zoonotic and human nature in surface waters. Contaminated water can be a problem to human health and the development of rapid methods for detection of microorganisms represent a valuable tool for the prevent its spread. Viruses as well others pathogenic microrganisms often occur in relatively low concentrations in environmental waters. Viruses object of our work were all enteric route viruses belonging to different taxonomic groups: Human enteroviruses (polio, echo and coxsakie), Human Hepatitis A and E (HAV, HEV), Norwalk viruses GI and GII (Noro GI, GII Noro), Human Rotavirus A, Mammalian Ortoreovirus, Human adenovirus F (Adv40, Adv 41) and Torque Teno virus (TTV).The aim of this work was to sampling and concentrate surface water from six different of European countries and identify by, molecular tools, the most representative viruses transmitted by oro-fecal route. The concentration of the sample was done by "filtration" in which the microorganisms adhere to a filter and an "elution" in which the microorganisms are resuspended through a process of reverse elution "backflushing". Through this system, 50 l of water sample are eluted in 1 liter of solution "backflush". The extraction of viral nucleic acids were performed with solution of guanidine. The RNA or DNA viral subjected to real time PCR reaction. The results obtained show a wide spread of enteric viruses in the different sites considered. Norwalk viruses (norovirus), Mammalian Orthoreovirus, and Adenoviruses are the most identified viruses. In water samples these viral species are positive in almost all the sites considered. Different results are obtained by the Hepatitis E virus (HEV) and Hepatitis A (HAV) where five and two out of fifteen were found positive respectively. The evaluation instead of Rotaviruses A and Human enterovirus (Polioviruses, Echoviruses, Coxsakieviruses)has produced negative results in all of the sites considered. Although it was not possible to establish the viability and infectivity of the viral species considered, the considerable presence of viral species in the sites under study poses a problem on the potential risk that contaminated surface water may pose to human health.

NITROGEN BALANCE FOR TEVERE RIVER BASIN: SOIL SYSTEM BODY MODEL APPLICATION (SSBMA)

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Nitrogen deficit or excess within ecosystems imply different problems. The scarcity of this key nutrient strongly affects agricultural productions while its abundance ("too much of a good thing") can determine water eutrophication and associated setbacks. At the beginning of 2014 a national initiative was launched to researchers dealing with nitrogen, including limnologists, ecologists, biologists, agronomists and hydro-geologists. Recently the INN (Italian Nitrogen Network) initiative has been built with the aim of elaborating a common methodology to evaluate the nitrogen budget at the watershed level. Here an example applied on the Tevere river is presented. The Model assess impacts related to anthropogenic pressures and land use on water quality arising from any N excess in environment. This approach evaluates N amount deriving from agriculture and livestock and the loss of N due to removal of crops, ammonia volatilization and soils denitrification processes. Any surplus of N is a metric of the potential N pollution in aquatic environments. The test area corresponds to the Tevere river basin area as the budget of N depends on the hydrological processes. The N amount estimate of the in the environment is evaluated using statistical and Geografic Information System (GIS) data for the studied area for the year 2010. All input data are converted into nitrogen units by means of site-specific, appropriate coefficients. Soil System Body Model evaluates only agricultural soils N input and output, ignoring all changes from one to another nitrogen form. Human wastewater contribution to N pollution is not keep into account into the model, cause the model is built only to compare agricultural and livestock N contribution. Than comparison between N surplus and N loose by agricultural system every year because crops take away, allows the assessment of the processes involving nitrogen loads during transport from the place of generation to the fluvial basin environment. Results show that 38% of N input comes from agriculture (synthetic fertilizers), 17% from livestock manure, 39% from biological fixation and only 6% from atmospheric deposition. On the other hand, N output come from 83% crop uptake, 10% NH_3 volatilization and 8% denitrification in soil. N Balance (input-output) amount 26,494 t per year 2010.

LABORATORY PERFORMANCES AND LIMIT OF QUANTIFICATION FOR MEASUREMENT OF PRIORITY SUBSTANCES

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Twenty-one Italian Regional/Provincial Environmental Protection Agencies (ARPA/APPA) are responsible for carrying out monitoring activities in the frame of Directive 2000/60/CE, while the Institute of environmental protection and research (ISPRA) is responsible for assuring the comparability of the analytical data produced by the ARPA/APPA laboratories as well as by other subcontracted authorities and/or organizations. Within the framework of the Proficiency Testing (PT) annual program, ISPRA carried out in 2013 a laboratory comparison on measurement of mass concentrations of some priority substances (PSs) in water. For all these PSs (i.e. Atrazine, Simazine, Chlorpyrifos, Fluorantene, Benzo[a]pyrene and Di-2-ethylhexyl phthalate-DEHP) the European Directive has established Environmental Quality Standards (EQS). Forty laboratories, belonging to Regional Environmental Protection Agencies (ARPA/APPA), received three mixture solutions (test items) of the selected compounds, produced by addition of suitable amount of certified reference materials. The first one, after reconstitution in water, has concentrations close to the EQSs. Before delivery, these test items were checked for homogeneity and stability. The second and the third test items have mass concentrations higher than EQSs. Participants were requested to measure mass concentrations in the sample obtained after reconstitution in water of the first test item and to measure directly the second and the third test item. The laboratories' performances were assessed by z-score, considering a 25% target standard deviation for all the priority substances. More than 80% of the laboratories have obtained acceptable z-score (equal to or below 2). Limit of Quantifications (LOQs) of measurement methods determined in the ARPA/APPA laboratories generally comply with the minimum performance criteria for Limit of Quantifications (LOQs) of European Directive; i.e. LOQs less or equal to 30%of the relevant EQSs. In nearly every case, the laboratories meet the required limits of quantification, with some exceptions for Chlorpyrifos. In respect to the revised ESQs, effective from 2015, there is still some work to do for Benzo[a]pyrene.

DEVELOPMENT AND EVALUATION OF REAL-TIME PCR AND ELIME ASSAYS TO REVEAL THE PRESENCE OF SALMONELLA IN IRRIGATION AND WASHING WATER OF CROPS

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Contaminated water sources used to irrigate and wash produce crops have been recently demonstrated to represent the main risk factors implicated in a large number of several outbreaks, due to pathogenic bacteria such as *Salmonella*. This problem is extremely relevant in some regions of southern Italy. Being the reference method for *Salmonella* detection (EN/ISO 6579) time consuming, there is the need for rapid and efficient methods to assure foodsafety and to protect consumers. Here we propose the development of two different techniques: a Real-Time PCR and an ELIME (Enzyme-Linked-Immuno-Magnetic-Electrochemical) assays to assess the presence of *Salmonella* in irrigation and washing water of crops.The Real-time PCR employs primers and a specific fluorescent probe able to amplify a region of the *ttrRSBCA gene,* conserved in all *Salmonella*serotypes, and a IAC to check false negative results. The standard curve obtained by plotting the mean Ct *vs* log concentrations of *S.* Napoli, showed good linearity from 10to 10^8 CFU/mL. The experiments were conducted analysing 70 salmonella and 38 non-salmonella strains. All salmonella serotypes tested were ttr-positive and all other bacteria yielded no amplification product. The ELIME assay is based on the use of magnetic beads (MBs), as support of a sandwich immunological chain, coupled with a strip of 8-magnetized screen-printed electrodes. The product of the enzymatic reaction is quickly measured by chronoamperometry at an applied potential of -100 mV for 120 seconds. Four different kinds of MBs were tested:Dynalbeads anti-*Salmonella*, Pathatrix anti-*Salmonella*, Pathatrix same day anti-*Salmonella*, Pan Mouse IgG MBs coated by us with a broad reactivity MAb anti-*Salmonella*.For each kind of MBs we evaluated the response of different *Salmonella* serotypes, using an optimized dilution of PAb-HRP, and non-target organisms.The best results in terms of current response towards *S.* Napoli and *S.* Thompson (recently isolated from vegetables grown in Italy) and selectivity were obtained using Pan Mouse IgG MBs, coated with MAb. Because we verified that the coated and blocked MBs were stable for several months, it was possible to develop a very simple and rapid assay, where the incubation between MAb/*Salmonella*/PAb-HRP occurs for 1 h in a single step. In the optimized conditions, calibration curves for *S.* Napoli and *S.* Thompson showed working ranges of 10^5-$2x10^6$ and 10^6-$5x10^7$ CFU/ml, respectively. Experiments on irrigation water of crops, experimentally contaminated with 1/10 CFU/mL of *S.* Thompson, have demonstrated the ability of both methods to reveal this pathogen.

VERY SAMPLE DYNAMIC MODEL (VSD+) AS A TOOL TO ASSESS IMPACTS OF CLIMATE CHANGE AND AIR POLLUTION ON BIODIVERSITY

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The availability of nutrients is one of the most important abiotic factor, which determine the plant species composition in ecosystems. Nitrogen is the limiting nutrient for plant growth in many natural and semi-natural ecosystem, especially of oligotrophic and mesotrophic habitat. The critical load is defined as "a quantitative estimate of an exposure to one or more pollutants below which significant harmful effects on specified sensitive elements of the environment does not occur according to present knowledge". Nutrient nitrogen pollution is a crucial issue in some Region of Italy, characterized by low critical loads and consequently high exceedance levels. This deposition above the threshold could be related to biodiversity loss. One of the methodologies proposed to estimate critical load in a single site is the Very Simple Dynamic Model (VSD+). VSD+ model has been developed on behalf of the Convention on Long-range Transboundary Air Pollution (LRTAP) as a tool to achieve the objectives identified by the Gothenburg Protocol evaluating soil chemical state not 'harmful' to a specific ecosystem and to reach the target of "no loss in biodiversity" for 2030. The model estimates the occurrence probability of species linked to a specified ecosystem. Site-specific VSD+ applicationsarehere presented with the aim to assess climate change and air pollution impactson Italian forest sites.The six selected forest sites are characterized by different climatic,ecological and topographical condition and are subjected to different air pollutant concentration and deposition. Occurrenceprobability have been estimated in the frame time from 1980 to 2030. In this way the impacts of acidifying and eutrophying air pollutants were evaluated for the past decades and for future scenarios, in order to verify the effectiveness of air pollutantreduction.

MICROBIAL QUALITY IN WATER AND FOOD

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Each year microbial contamination in water and food kills thousands and sickens millions of people and is a major global health concern. Presence of indicator bacteria such as *E.coli*, total coliforms or thermotolerant coliforms in the water indicates potential presence of a fecal contamination and then again presence of pathogen organisms, e.g. parasites like *Giardia lamblia* and *Cryptosporidium parvum* and the *Norovirus*. Traditional testing for indicator bacteria in the laboratory is time-consuming (1 up to 3 days) and are retrospective tools to alert the public when the water is contaminated. Hence, at the time when the test results are ready, the public has already been exposed to the health hazard. Combined with not so frequent testing of the water, a contamination episode might not be detected until the public are starting to get sick. Based on this knowledge there is a need for methods that are faster than the traditional methods, and additionally give the user possibility to take out more frequent samples. Colifast AS, represented in Italy by ECOTOX LDS, has developed a technology for analyzing indicator bacteria in water, raw water, fresh water, seawater, drinking water, bottled water and process water. The Colifast technology takes microbial methods used in the laboratory, and combine the patented Colifast growth media with fully automated analyzing monitors and place them at site. The instruments send the results via industrial interface (relays), GSM and LAN as well as visual and audio alarms on site. This technology, related to the At-line automated remote monitor, has been submitted to EPA's ETV Program. The technology detects total coliforms, thermotolerant coliforms, E.coli and Pseudomonas aeruginosa in water samples. Each of these bacteria has group-specific enzymes that hydrolyze complementary substrates that is present in the selective Colifast growth medium. Fluorescence from the hydrolysation product, a fluorugenic subtrate, is measured by the Colifast Instrument. The fluorescence intensity is a function of the number of target bacteria. The Colifast technology enables early warning of bacterial contamination as the monitors alarms the user as soon as a positive sample is detected. The rapid results give the operator extra time to act and take operational and quality control decisions. In addition Colifast AS also have a Field Kit with battery driven analysing unit and a test for spoilage bacteria in fresh marine fish, Colifast FAST FISH TEST.

SALMONELLA INFECTIONS: INTEGRATED MEDICAL, VETERINARY AND ENVIRONMENTAL SURVEILLANCE IN ITALY

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Temperature increases and changes in rainfall patterns have an impact on the persistence and patterns of occurrence of bacteria,viruses, parasites and fungi and their corresponding foodborne diseases. Such changes also have an impact on microbial ecology and growth, plant and animal physiology and host susceptibility which may result in the emergence, redistribution and changes in the incidence and intensity of plant and animal diseases and pest infestations, all of which could impact foodborne diseases and zoonoses. Recognizing, understanding and preparing for the impacts of climate change further highlight the need to promote interdisciplinary approaches to addressing challenges affecting food safety given the inter-relationships among environmental impacts, animal and plant health impacts and food hygiene. These inter-relationships are further complicated by the broader public health implications of climate change as well as the food security implications. Salmonellosis is a major cause of human bacterial gastroenteritis and the second most reported food-borne zoonosis in the European Union (EU), after campylobacteriosis. Humans can become infected with Salmonella from several sources mainly food of animal origin but also vetetables and via different pathways, including direct contact with live animals and environmental transmission. In this framework a laboratory-based surveillance system is a useful tool to monitor the diffusion and the emergence of infectious agents in human infection, in animal reservoirs, in food vehicles and in environmental sources. For Salmonella infections, the italian surveillance, EnterNet, collects Salmonella strains isolated from human infections and environmental sources and share epidemiologic and microbiologic data with a similar veterinary network, EnterVet, which collects information and strains isolated from animals and food of animal origin. In addition the two italian networks follow the evolution of the surveillance activities undertaken at the international level. Data from the last 5 years of surveillance show the prevalence of Salmonella serovars such as S. Typhimurium in human infection, animal and foodstuff as well as from surface water confirming the ubiquitariety of this serotype. On the other hand other serotype such as S. Napoli responsible each year for 3-4% of human salmonellosis are isolated from human infection and environmental sources but not from farmed animal or foodstuffs suggesting a wild reservoir for this serovar and an important involvement of environmental condition for its maintenance in water ecosystems.

A RAPID AND INNOVATIVE ANALYTICAL APPROACH TO VERIFY THE PRESENCE OF *SALMONELLA* IN SURFACE WATERS

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Contaminated waters as well vegetable should be considered important way for the transmission of Salmonella to humans. Pathogenic microorganisms often occur in low concentration in surfacewater and in vegetables. Cultural methods, currently adopted to assess the presence of *Salmonella spp* in water, required long analysis times. The aim of the present work was the development of a rapid method, based on the use of Real-Time PCR, for the detection of Salmonella spp bothraw and concentrated waters of rivers and lake.The proposed Real-Time PCR employs primers and a specific fluorescent probe of the *ttrRSBCA gene,* conserved in all *Salmonella* serotypes. Water samples of five sites were studied: three sites were located in *Latium* Region, two on Tiber River (Castel Giubileo and Mezzocamino) near Rome and one lake (Albano); others two rivers were located on Chienti (Piediripa) e Potenza (Fonte di Brescia) of Marche Region. Both fifty and 3 liters of raw water were sampling on each site and transported and stored at 4°C until analysis. 50L of water were concentrate into 1 liter, while one liter of raw water were filtered using Membrane Filter (MF) technique. In order to establish the minimum pre-enrichment time, necessary to reveal the presence of *Salmonella* in water, all filters and an aliquot of concentrated water for each sample were place and were incubated with BPW. The aliquots, during the incubation time, were collected at 0, 2, 4, 6, 8, and 24 h and submitted to DNA extraction using a commercial kit and Chelex 100 and real time PCR.Further to reduce the time of incubation, an aliquot of the same samples was treated with immunomagnetic particles anti-Salmonella, that allows the simultaneous separation and specific concentration of the bacterium from samples. The results obtained in both case showed that Real-Time PCR is able to detect *Salmonella* after 8 hours of incubation and the results showed a concordance of 100% with the reference method EN/ISO 6579. Finally, the Ct values were similarly both concentrated and raw water samples. It is suggest that the analysis of raw water is representative of site studied. On the basis of these findings, in order to trace the origin of this contamination the development of a rapid method for the detection of *Salmonella spp* in surface water should be consider crucial for prevention and control of infection due to this microorganism.

ISOLATION AND IDENTIFICATION OF POTENTIALLY TOXIC AMPHORA COFFEAEFORMIS (AGARDH) KÜTZING IN TRANSITIONAL WATER SAMPLES OF THE TYRRHENIAN COAST (ITALY)

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Amphora coffeaeformis (Agardh) Kützing is a benthic diatom, that lives mainly in brackish and marine ecosystems. This diatom has been reported as a potential producer of domoic acid (DA), a low molecular weight amino acid derivative acting as a neurotoxin agonist at the glutamate receptors. DA was first identified in Canada in 1987, when four people died after intoxication with mussels containing diatoms. In the present study, twenty-two samples were collected from transitional waters (coastal lakes, river delta and channel estuaries) of the Tyrrhenian coast of Lazio Region (central Italy) during two seasons in 2011 (spring and summer). Sampling was performed by scraping stones, macrophytes, silt and sand. Following treatment with hydrogen peroxide and hydrochloric acid, diatom cells were analyzed and identified at species level using light microscopy (1000x). The morphological analysis of diatom frustules showed that a specific diatom belonging to the genus *Amphora*, named *Amphora coffeaeformis* (Agardh) Kützing, was identified in lentic and lotic transitional waters investigated, with a salinity higher than 4 ‰. This seemed to be the main environmental factor that influenced its distribution. Subsequently, pure cultures of *A. coffeaeformis* were kept under laboratory conditions at 24±1 °C and with light/dark cycles of 15 and 9 hours, respectively. Liquid chromatography coupled with mass spectrometry system (LC-MS), was employed to investigate the presence of DA in culture samples. In addition, an ecotoxicological assay based on *Vibrio fischeri* with Microtox test was performed. The aims of the present study were: i) to investigate the presence of toxic *A. coffeaeformis* species in transitional water ecosystems along the Tyrrhenian coast of central Italy, ii) to isolate and obtain pure cultures of this diatom species, iii) to evaluate the presence of the algal toxin domoic acid in diatom cultures. The mechanism of domoic acid production still remains unclear. Previous reports suggest that it could be influenced by several environmental factors such as physical, chemical and biological parameters. The laboratory cultures described in this study will be used to evaluate the production of this toxin by *A. coffeaeformis* under the effect of environmental stressors.

PRODUCTS OF INDUSTRIES: WATER TREATMENT SYSTEMS AS SAFEGUARD AGAINST PATHOGENS

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Water is a major vehicle ofdiseases spreading, as men use itdaily to satisfy physiological needs, for recreational purposes, for production activities. In the water it can be found both chemicals andbiological contaminants.Some products of industries using water in the manufacturingprocess may have direct or indirect impact on human health (e.g. food, food supplements, drugs, medical devices). Water is largely adopted for manufacturing drugs and medical devices, as excipient, or used for reconstruction of products, during synthesis, production of finished products or as cleaning agent for rinsing equipment, primary packaging materials, etc. The microbiological quality of water is particularly critical, and companies are required to invest large resources to ensure it. The specifications of water for pharmaceutical use are reported in the Italian, European and American Pharmacopoeia, and are constantly updated. Three grades of water are reported in the Pharmacopoeia: purified water; highly-purified water and water for injections. Moreover, drinking water, although not reported in Pharmacopoeia, must also be considered, as frequently represents the incoming water for the treatments mentioned above. Drinking water is strictly controlled by national regulations. Specific rules or guidelines are still lacking for medical devices and usually the rules developed in pharma are applied. The grade of water used should take into account the nature and intended uses of the finished product and the stage at which the water is used. The guidelines of the European Medicines Agencyon pharmaceutical preparations, also applicable to medical devices, provide some examples:

- purified water to be used for all non-sterile and non-injectable preparations, for sterile preparations for topical, oral, nasal, ophthalmic and ontological use;
- water for injections to be used for sterile devices for parenteral use, for haemofiltration solutions, solutions for dialysis and irrigation, for injectable devices.

Regarding the microbiological quality of water, the guidance documents also provide the limits to be followed. In particular, the European Pharmacopoeia includes following mandatory microbiological limits (Bacteria):100 c.f.u/ml for purified water and 10 c.f.u./100ml for water for injections and highly-purified water. On the contrary, the United States Pharmacopeia doesn't report these limits as mandatory, but indicates them in the form of recommendation. Using validated and controlled water treatment systems and developing rapid and efficient methods to monitor the presence of emerging and re-emerging pathogens is warranted to obtain water with required quality attributes, and to ensure the safety of finished products for human health.

CLIMATIC AND ENVIRONMENTAL CHANGES MAY FAVOUR THE INTRODUCTION AND THE ESTABLISHMENT OF MOSQUITO BORNE DISEASES IN ITALY

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During the last decade, the slow but constant rise in the average temperature of the earth, the environmental changes induced by human activities and, in general words, the quick increase in the globalization, have raised the concern about the possible introduction (or re-introduction) of tropical Vector Borne Diseases (VBD) in European Countries. Due to its peculiar geographic position, Italy is considered one of the most "at risk" areas, in particular for Mosquito Borne Diseases (MBD). Among the arthropods vectors of human diseases, mosquitoes appears to be the more susceptible to climatic changes, being part of their life dependent on the availability and quality of the water. The importance of the mosquitoes as disease vectors is related, above all, to the transmission of malaria. Despite malaria was eradicated in Italy in the 1950'-1960's, the main vector *Anopheles labranchiae* survived to the DDT treatments and quickly re-colonized part of the territory. Recently a 5-year study aimed to assess a possible reintroduction of malaria in Italy was performed by the Authors. The results have excluded the return to an endemic situation; however the occurrence of sporadic, isolated cases of autochthonous malaria *(Plasmodium vivax*) between May and October cannot be excluded in those areas where *An. labranchiae* may seasonally reach high densities. A more real risk for Italy is represented by other MBD due to arbovirus transmitted by *Culicinae* mosquitoes. These species and other exotic vectors of possible introduction (i.e. *Ae. aegypti, Ae. japonicus),* are all characterized by a great ability to adapt to different environment and climatic conditions and to quickly become established in new areas. Particular relevance deserve the two most widespread species: *Aedes albopictus,* (the"tiger mosquito") introduced in 1990 by tire trading and quickly spread across the whole country, was responsible of the outbreak of Chikungunya (CHIK) virus occurred in Emilia Romagna in 2007 starting from an imported case; *Culex pipiens*, the most common indigenous mosquito, has represented since 2008 the main vector of West Nile (WN) Disease, a zoonosis recently introduced by migratory birds and that is quickly going to become endemic. Recently, we investigated the vector competence of several field collected Italian populations of both species by experimental infection: *Ae. albopictus* with Dengue, CHIK and WN viruses and *Cx. pipiens* with WNv only. All tested populations became infected, showing different degree of competence. However, the influence that climatic and environmental changes may have on vector bionomics and, as a consequence, on the real impact of new MBD on human health are still matter of study.

COULD PLASMODIUM VIVAX MALARIA RE-EMERGE IN TEMPERATE CLIMATE ZONES OF EUROPE?

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P. vivax is currently the most common causative agent of human malaria since this parasite is present not only in tropical or sub-tropical areas of the world but also in many temperate zones, such as the Southern states of the former USSR, Caucasian region and Turkey, with an estimated number of infections of 390 million per year. Although P. *vivax* infection is rarely fatal, malaria due to *P. vivax* is a highly debilitating disease with a heavy socio-economic burden on the communities living in endemic areas. Recent cost burdens are estimated to be 1.4-4 billion of US$ per year. Despite these problems *P. vivax* is still considered a "neglected" human parasite because of large gaps in knowledge of its biology. The relapsing behavior of this parasite and the identification of Asian strains resistant to chloroquine, which is still the antimalarial of choice for the treatment, contribute to make the control of *vivax* malaria increasingly difficult. New worrying features about this parasite are also the transmission among Duffy blood group–negative population and the occurrence of severe or even fatal infections (ARDS, splenic rupture, cerebral malaria). Moreover, climatic changes with associated extension of seasonal transmission areas could become a cause for concern of P. *vivax* reintroduction in malaria-free areas. Malaria was officially eradicated in temperate regions of Europe at the beginning of 70's. The last countries to be declared malaria free in the European region were Italy (1970), Portugal (1973) and Greece (1974) due to the fact that, in these countries, sporadic local cases of *P, vivax* malaria occurred along the decade preceding the eradication dates. From year 2008 to year 2012, three European areas (Ebro Delta, Spain; Camargue, France; Maremma, Italy) were extensively investigated for the risk of malaria reintroduction, with particular regard to the assessment of vulnerability of the study areas. Recently, an outbreak of malaria was recorded in Greece, with several dozens of cases reported since2011, reflecting the fact that this parasite is able to re-emerge in temperate areas in Europe where it had been eradicated long time ago.

INTERLABORATORY EVALUATION OF A COLONY HYBRIDIZATION METHOD FOR *VIBRIO PARAHAEMOLYTICUS* ENUMERATION

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The development of a colony hybridization method for the enumeration of *Vibrio parahaemolyticus* was recently described. The method is based on a cultural step, with the inoculum of the samples on a non-selective agar medium, and on a molecular assay, in which colonies are transferred to a membrane, bacterial DNA is covalently linked to it, and enumeration of *V. parahaemolyticus* colonies is performed using DIG-labeled oligoprobes and colorimetric detection. The method was evaluated on four experimentally contaminated seafood matrices (shellfish, finfish, crustacean and cephalopods) for linearity, accuracy and repeatability, and was validated in-house on naturally contaminated shellfish. To assess the performance of the method in reproducibility conditions (different operators and equipments), a small-scale interlaboratory trial was performed. For each of the four participants, three shellfish homogenates were prepared with different *V. parahaemolyticus*inoculum levels (L_0<10 *c.f.u.*/g, L_1≈300 *c.f.u.*/g, L_2≈3000 *c.f.u.*/g). Samples were shipped under refrigerated conditionsto the four laboratories and analysis were performed within 24 hours. All materials and regents required for the analysis, including the controls, were supplied together with the samples. Three laboratories were able to complete the analysis according to the protocol and reported results. L_0 samples were correctly identified as below the detection limit of the method (<10 *c.f.u.*/g) by all participants, while the coefficient of variation of the results for L_1 and L_2 levels was respectively 13% and 14%. In total, the calculated standard deviation of reproducibility was 0.058 log, with a corresponding reproducibility limit of 0.161 log. In conclusion, the results of the interlaboratory study demonstrated the good performance of this colony hybridization in reproducibility conditions.

GONADAL DISORDER IN THE THINLIP GREY MULLET (LIZA RAMADA, RISSO 1827) AS A BIOMARKER OF ENVIRONMENTAL STRESS IN SURFACE WATERS

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Fishes are among the most widely used bioindicators for aquatic ecosystem "health" assessment, because they may respond to environmental stresses at the different levels of biological organization, from sub-cellular to community level. Sexual disorders in fish have been linked to exposure to chemical contaminants in many aquatic ecosystems around the world and increasingly used to assess the effects of pollution on aquatic ecosystems. Among compounds of human origin, the *Endocrine Disrupting Chemicals* (EDCs), derived from the degradation of surfactants and commonly detected in the discharge of sewage treatment plants, have effects analogous to sex steroids and can induce degeneration of gonadal tissues, causing reduction in gonad weight and volume, disorders in gonadal maturation, increased occurrence of intersex, ovotestis, testicular oocytes, gonadic atresia. Hence, the anatomical and histological status of gonads on wild fish may be used as useful biomarker to consider the presence of these contaminants in surface and coastal waters. The aim of this study was to appraise the use of gonadal alterations in the thinlip grey mullet (*Liza ramada*) as a biological indicator in assessing the health of aquatic ecosystems, such as urban river ecosystems exposed to sewage discharges. For this purpose, reproductive status and presence of gonadal alterations were studied in 206 mullets collected from two sites on the low course of the Tiber River, downstream of a large urban sewage treatment plant and in the estuarine area, and from an uncontaminated pond considered as reference site. Intersex and irregularly shaped gonads were observed in 20.8% of the mullets from the most polluted site, and intersex gonads in 10.3% of those from the estuarine area. No alterations were detected in the fish from the reference site, which also showed well defined stages of gonadal development, the highest seasonal values of gonado-somatic index (GSI) and the lowest values of epato-somatic index. Conversely, only poorly defined stages of testicular and ovary development were observed in fish from the two polluted river sites. The results of this study suggest that *L. ramada* may represent an excellent sentinel species in environmental risk assessment, and support the use of gonadal alterations of this species as a biomarker for extensive monitoring of pollution in lower stretches of rivers and estuarine areas.

ASSESSING EFFECTS OF ANTHROPOGENIC STRESS ON AQUATIC ECOSYSTEMS USING A MULTILEVEL INDICATORS APPROACH: THE CASE STUDY OF SACCO RIVER (*LATIUM*, ITALY)

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Anthropogenic pressure on aquatic ecosystems may cause changes in their structural and functional characters as well as negative impacts on human health. Thus effects of environmental contamination may be difficult to assess if only few descriptors are taken into account and only some matrices (e.g. water and sediments) are considered. In this context, over the last ten years a growing need for developing new biological approaches aimed at assessing effects anthropogenic stresses on aquatic ecosystems has been strongly highlighted, as indicated in recent European Community legislation (i.e. Water Framework Directive 2000/60/EC). This work is aimed at demonstrating an approach that considers multilevel indicators of fish health: i) genotoxic potential (micronucleus test in erythrocytes); ii) sexual disorders (gonadal tissues); iii) morphoanatomical disorders (skeletal anomalies); iv) community structure alterations. The case study deals with part of the Sacco River, between hill spring area and lowland stretches not far from Rome, where a serious alarm for the high toxicity risk has been declared by Regional Environmental Agencies since 2005, mainly attributable to the organophosphate pesticide HCH (lindane). Samples have been seasonally collected in four sites, representative of different environmental situations. Preliminary results from the erythrocyte micronucleus tests, carried out on specimens of four fish species (two benthic and two from the water column) have shown a significant increase of MN frequency in the individuals collected in polluted areas relative to those collected in less polluted upstream areas, with maximum frequencies detected in benthic and benthivourous species. High frequencies of skeletal anomaly, detected both in juvenile and adult fish, highlighted a diffuse stress on fish across all the study area. Ecological quality status assessed by applying Fish-based Decision Support System (FiDeSS) has shown that sampling sites did not attain a “good” ecological status, with the worst evaluations downstream of a local industrial area. Our results highlighted a weak concordance among evaluations obtained from the different bioindicators we considered, thus showing that bioindicators at different level of biological organization may not agree with each other, confirming the need for an integrated approach in ecological and health status assessments of river ecosystems.

ENVIRONMENTAL MONITORING: THE CASE STUDY OF PIETRA DEL PERTUSILLO LAKE (ITALY)

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The Pietra del Pertusillo lake, located in Basilicate Region (Italy), is an artificial lake derived from the barrage of the Agri river. The lake covers an area of 75 km2 and provides a multiple use of water resources such as hydroelectric power, irrigation and is one of the starting points of the Apulian aqueduct. The study of the presence of fecal origin microbiological species in this lake is therefore an important tool for the protection of human health of the population directly or indirectly using this water reserve. Viruses as well others pathogenic microorganisms often occur in relatively low concentrations in environmental waters and the development of effective methods for water concentration and identification of pathogenic organisms represent valuable tools for prevent its spread. The purpose of this work was to determine the microbiological quality of Pietra del Pertusillo lake through the detection of fecal bio-indicators as Entorococci, E. coli and enteric viruses in large volume of water. 50 liters of water were sampling in the center of lake. The concentration of 50l was performed by filtration, in which the microorganisms adhere to a filter, and an elution, in which the microorganisms are resuspended through a process of reverse elution "back flushing". Through this system, 50l of water sample are eluted in 1 liter of "back flush" solution according to the methodology developed in the "μAQUA" project. Detection of E. coli and enterococci was performed on aliquots of back flush solution using the membrane filter (MF) technique, after which the membrane is transferred to the surface of a selective agar plate. For virological analysis, the back flush solution was subjected to extraction of viral nucleic acids using guanidine. RNA and DNA were quantified and used to perform real time PCR. Negative results, no growth, were obtained both E.coli and enterococci. Similar results was obtained for virological data. The detection of Human adenovirus 40 and 41, Hepatitis A, Hepatitis E, Noroviruses, Rotaviruses, Mammalian Orthoreovirus, Human enterovirus (Polioviruses, Echoviruses, Coxsakieviruses) has produced negative results in all examined samples. This work represents a first application of techniques for the concentration of large volumes of water, to research microorganisms, in environmental monitoring according to the Regional Environmental Protection Agency.

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