Instrumentation for continuous monitoring in marine environments

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***Abstract*- Continuous, high frequency data is a useful source of information for the understanding of seasonal chemical and biological changes in marine environments. They are useful to estimate nutrient dynamics, primary and secondary production as well as to assess C, N, P fluxes associated with biogeochemical cycling. More and better water quality data is needed to calculate Maximum Permissible Loading of marine environments as well as inland waters and we need better data to assess trends, to determine current status of waters and their impairments, and to test water quality models. For a long time these requirements were not met with satisfactorily due to the absence of suitable instrumentation in the market.**

SYSTEA has tried to bridge this gap since ten years with the development of several field analyzers (NPA, NPA Plus and Pro, DPA series and latest is the WIZ-probe) and participating in several R&D European projects (EXOCET/D, WARMER) where has been proven the ability to build reliable and efficient in-situ probes which are now commercially available. The choice to work in collaboration with scientific institutions specialized in marine ecosystem study has been made very early by SYSTEA and is actually the only company able to offer a complete range of in-situ probes for continuous nutrient analysis, using its exclusive µ-LFA technology fully developed by SYSTEA in collaboration with Sysmedia S.r.l with remote management capabilities. These innovative technical solutions allow deploying their DPA probe down to - 1500 m depth, maintaining a high level of accuracy and robustness as proved during the European project EXOCET/D in 2006.

The WIZ probe is the latest development of SYSTEA, the state of the art portable "in-situ" probe, to measure up to four chemical parameters continuously in surface waters or marine environments. The innovative design allows an easy handling and field deployment by the user. WIZ probe allows, in the standard configuration, the detection at trace levels of four nutrient parameters (orthophosphate, ammonia, nitrite and nitrate). WIZ probe autonomously manages the well tested spectrophotometric wet chemistries and an advanced fluorimetric method for ammonia measurement. Analytical methods have been developed for several other parameters including silicates, iron and trace metals which are available on request. Results are directly provided in concentration units; all measured values are stored with date, time and sample optical density (O.D.). The same data are remotely available through a serial communication port, which allows the complete probe configuration and remote control using the external Windows® based Wiz Control Panel software.

Keywords: Continuous nutrients monitoring, in-situ multiparametric analyzers, microLFA technology, WIZ probe

I. INTRODUCTION

Eutrophication is a global phenomenon caused by the excessive phosphorous and nitrogen loads to water-bodies. Accelerated eutrophication as a process of water quality deterioration is most often due to human influences, including nutrient load from both point and non-point sources [1, 2]. The increased nutrient loading is a result mainly of cities, settlements, recreational pressures, industry, deforestation, agriculture, erosion, poor watershed management and from atmospheric loading. In the marine environment, the waters that are readily subject to eutrophication are those that have a limited interaction with the pelagic waters adjacent to them. Due to rapid eutrophication of coastal and inland waters, in-situ monitoring of water quality with particular emphasis on nutrients is a global priority theme in water analysis.

In Europe, continuous monitoring of large rivers and other inland waters dates back to early seventies. The development of sensor networks were intensified during early nineties through integrated river basin management programs (IRBM) resulting their introduction to large number of transboundary rivers and lakes [3]. The European water framework directive (WFD) which came into action in Oct. 2000 extends the requirement for close monitoring of the coastal waters because all land-based inputs of pollutants pass through the coastal zone to the open waters [4, 5]. At present coastal waters are routinely monitored, mostly as a requirement for bathing water monitoring in summer months. However, continuous long term nutrient sensing would bring accurate information for calculation of maximum permissible loading (MPL) limits and diffuse nutrient inputs that are required to control rapid deterioration of water quality in both coastal and inland waters.

These different types of water bodies under consideration show a wide range in nutrient distribution. The common nutrients of main concern for in-situ and continuous analysis in the aquatic environment are nitrogen, phosphorous and silica as they could accelerate eutrophication processes [1,2] or can limit primary productivity in oligotrophic waters [6,7]. In the stratified open ocean in the pelagic zone where oligotrophic conditions prevail, nutrient concentrations range from low nanomolar in the surface waters (e.g. ultra-oligotrophic zones) to micromolar at depth or in some eutrophic coastal regions and brackish estuaries. Nitrate and phosphate distribution span up to five orders of magnitude from low nanomolar to micromolar depths in the open ocean [8]. Development of monitoring techniques and instrumentation for in-situ analysis and continuous monitoring of such diverse environments is a challenging task.

Recent developments in optical sensor technology provide advanced analytical tools [9] for continuous assessment of nutrient concentrations in the aquatic environment. The requirement of new instruments for in-situ and continuous nutrient monitoring in these diverse environments has encouraged the development of a series of novel on-line analyzers using newly emerging technologies during the last two decades [8-12]. Despite these developments, standard wet-chemical analysis, offer the most suitable analytical techniques to detect very low concentrations of nutrients in marine and fresh water environments [8, 10,17, 18, 30,31].

Historical development of in-situ probes for continuous-monitoring of nutrients

The field application of laboratory methods for nutrients measurement has been a difficult task due to the complexity of their automated application for on-line analysis. There is a need to integrate other demands such as sampling, sample processing, calibration, data generation, results analysis and report generation controlled by the instrument. When they are used in widely distributed sensor networks, require new strategies to deal with calibration, data validation and device diagnostics. The above mentioned features have been gradually introduced in several in-situ analyzers developed during the last two decades and they have been deployed in a wide variety of marine environments.

In 1993 three prototypes of Micromac-1000 analyzers for on-line nutrient analysis were developed using a novel technology called ‘Loop Flow Analysis’ (LFA) and successfully deployed on marine platforms in the Mediterranean [14-16]. These LFA analyzers were first used to monitor trace concentrations of nutrients using standard wet-chemical methods [17, 18]. This new technology revolutionized the continuous monitoring with the introduction of miniaturized, portable, stand alone reliable models that could be used unattended efficiently in both on land and sea. The recent Micromac models have the capability to measure up to four nutrient parameters (phosphates, nitrates, nitrites and ammonia) or other combinations (e.g. silicates, iron, cyanide or trace metals) and the LFA technology is patented in Europe and USA [19]. The development of the first generation in-situ nutrient probes using LFA technology was started in year 2000 and several were used for research projects in the Mediterranean [20] and rivers in the U.K [21]. The participation in the “Extreme ecosystem studies in the deep Ocean” research project (EU-STREP, EXOCET/D) [22, 23] led to the development of second generation LFA analyzers (µLFA), a new series of analyzers represented by NPA-Plus, NPA-Pro and the DPA. During the EXOCET/D, MoMARETO cruise (22,23), a DPA installed on the Remote Operating Vehicle (ROV Victor 6000) was used to measure total sulphide and Iron-II or total iron in deep sea hydrothermal environment at 1650m depth (24).

During the last years several instruments from the NPA and DPA-series have been installed in coastal marine buoys and platforms in the littoral zones of the Mediterranean, Arabian Gulf and the South China Sea for research activities and surveillance monitoring. Our objective in this paper is to present some outstanding features of the second generation µLFA analyzers, and the advanced features of the ‘WIZ’ the recent addition to this series.

II. MATERIALS AND METHODS

Instrument description

The Deep-sea Probe Analyzer (DPA) is designed to perform sequential measurement of four common nutrient parameters, namely dissolved orthophosphate, ammonia, nitrite and nitrate, which are normally measured during field studies. Other possible parameter combinations include silicates, iron , and other metals).

It is composed of two identical PVC cylinders (diameter 140 mm, height 785 mm) submersible up to 30 m depth, of which one is the analytical and the other the reagent container. The analytical and reagent container communicate with each through an umbilical connecting cord carrying ten delivery lines for reagent and calibration solutions. A specially designed hydraulic multiconnector speeds-up reagent replacement. On the cover of the analytical cylinder are hydraulic connections for water inlet and outlet, waste, MilliQ® de-ionized (DI) water and a water proof connector for the external 12 Vcc power supply and RS-232 serial connection.

The internal layout of the analytical compartment is optimized to install the electronics (sealed electronics assembly composed of an auxiliary board, the CPU and I/O boards) and the hydraulics. The hydraulics include: peristaltic pump, the micro Total Analysis System (µTAS) hydraulic manifold, the spectrophotometric flow-cell (length 20 mm), a quartz fluorimetric flow-cell

(length 10 mm), a specially designed mixer; a heating unit; the UV-NO_3 reducing device. All the hydraulic connections are with 1.6 mm Teflon tubing, Teflon nuts and ferrules.

The spectrophotometric optoelectronics is based on two LEDs (525 nm and 880 nm; for nitrite and phosphate detection resp.) and a solid state detector mounted on both sides of the flow-cell through a fiber optic cable; a second solid state detector is mounted on the back of the emitting LED in order to provide a reference signal. The flourimetric detection of ammonia is performed at 370 / 420 nm (excitation/detection).

The firmware runs in a multitasking environment and it is programmable through the connection to an external PC with RS-232 serial port. The analytical cycle is performed by the execution of a predefined analytical sequence of macro-commands. The user interacts with the analyzer in lab or in the field, working with a Windows XP® based software on the PC (NPA/DPA control panel Sysmedia Srl, Italy), which allows the complete remote control and management by a GSM/GPRS device.

Each measured value is stored inside the internal memory with date, time and the optical density (OD) start and OD end readings; the analyzer is able to internally compensate the intrinsic water color for each sample, measuring the OD before the injection of the color reagent (spectrophotometric methods) or the fluorogent (fluorimetric method).

Fig. 1 Hydraulic assembly overview

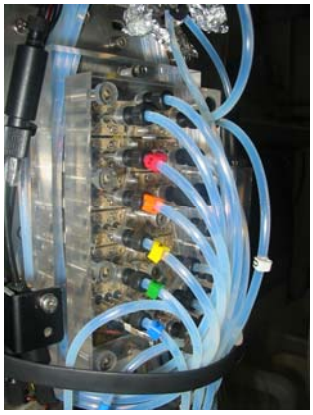


Fig. 2 The µLFA hydraulic manifold

***m**icro **L**oop **F**low **A**nalysis (µLFA) technology overview*

micro **L**oop **F**low **A**nalysis (µLFA) is an analytical technology for autonomous management of a microfluidic system to handle complex analytical methods using a batch principle[19]. In a 4 ml volume hydraulic loop, preliminary filled with DI water after the end of the previous measuring cycle, the water sample is collected and then the required reagents are sequentially injected and mixed, performing the specific conditioning procedure needed for an analytical reaction. As soon as the measurement is performed, the hydraulic circuit is washed with DI water. The process can be repeated again with the same method or with a different analytical procedure.

Analytical cycles

The schematic diagram of a four parameters **m**icro **L**oop **F**low **R**eactor (µLFR) is shown in Figure 3. Valves S/L (V8 and V7) allow alternatively to open the µLFR to pump in water sample, standard solution, DI water or to keep the µLFR closed in LOOP position, to allow mixing of reaction products. The reagents or calibration standards are introduced in to the µLFR using the movement of the pump; a mixer device creates turbulence inside the loop circuit, speeding up the mixing phase.

The sampling is enabled with valves S/L opening up and activation of the pump P; the water sample is pumped trough VK6, V11, VK5 and V7 inside the loop, passes through the mixer, the colorimetric flow cell and than flows out to the waste through the opposite section of the valves S/L. The system reads and stores the Sample Blank value that will be subtracted from the final OD of the reaction product.

To inject any reagent into the µLFR, the specific valve connected with the reagent bag is opened for a defined time while the pump is moving and the loop is open to ensure the exact quantity of volume introduced inside the loop. Valves S/L are then repositioned back to the LOOP position and the pump P is activated, to allow a fast mixing of sample and reagents. The reaction takes place all over the loop, including the flow cell allowing the continuous monitoring of the reaction development; at the end point O.D. is read and stored.

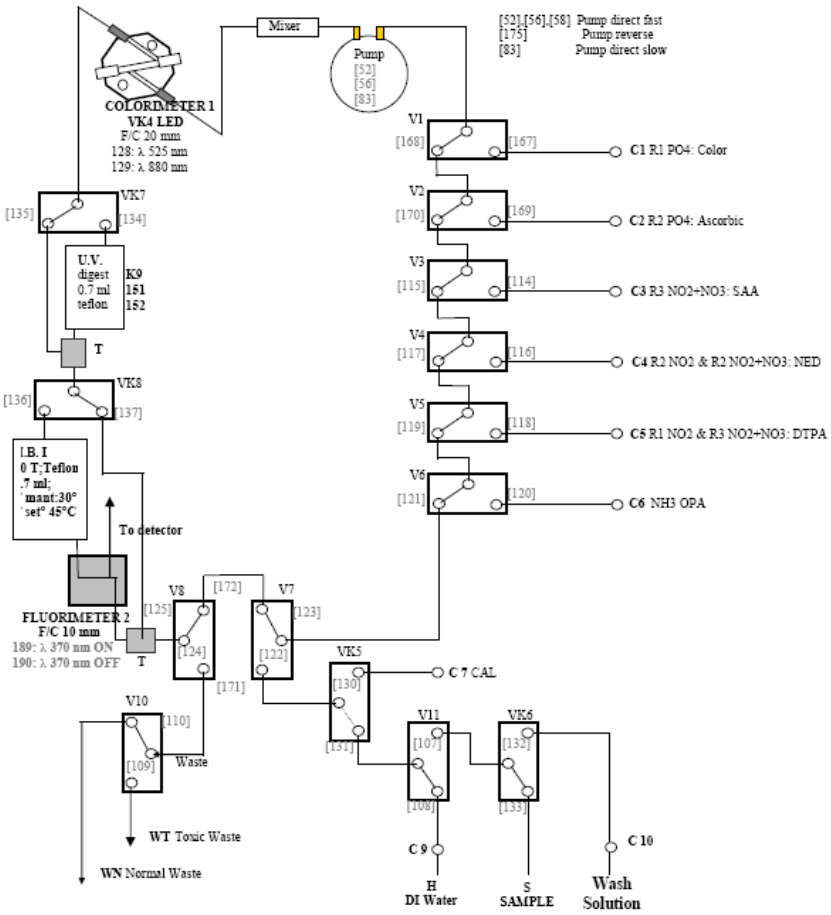


Fig. 3 μLFR schematic hydraulic diagram

To activate the wash cycle to clean up the circuit, valves S/L are switched on with all other valves in off position, allowing the introduction of DI water or the sample either for cleaning or rinsing. Pump P is activated in direct mode, water fill up μLFR washing hydraulics and then the analyzer goes to stand by, ready for a new run.

The calibration cycle allows the instrument recalibration using a stock standard, 20 times concentrated than the analytical method's full scale requirement. μLFR is filled with DI water and then a defined amount of stock standard solution is pumped inside the circuit as follows: with valves S/L and VK5 on, the pump P is activated; stock standard solution is pumped through VK5 and V7 inside the loop reactor. μLFR is closed and the injected stock solution is diluted with the DI water contained in the loop and the rest of process is repeated as previously described.

The μLFA technology allows the multiparametric sequential management of different analytical methods in the same circuit, with the possibility to operate in coastal waters [15,16,20] as well as deep sea, in the latter case using a pressure compensation system [22-24].

The hydraulic circuit can be easily washed, with the possibility to apply automatic dilution of the sample. The use of a loop configuration allows an easy management of bubbles problems for certain methods like phosphate analysis or sample conditioning devices like UV photo-reductor. Similarly auxiliary heating units can be integrated in the circuit and could be managed as required according to the analytical method.

Recent advances in the μLFA technology (WIZ probe)

The WIZ probe is the latest development and it can be actually considered the state of the art portable "in-situ" four parameter nutrient probe, with capability of measuring phosphate, ammonia, nitrite and nitrate sequentially, similar to the DPA analyzer, with same accuracy. The miniaturization of the instrument has increased the ease of handling and the compact cylindrical design allows the deployment similar to conventional in-situ water quality monitoring probes. The PVC single body is 140 mm diameter

and 720 mm in height, including the reagents container. The former two chambered DPA has been transformed into a less bulky, single unit, without sacrificing accuracy and performance.

The smaller reactor (1.5 mL µLFR) enables an extremely low consumption of reagents and calibrants: in Table I reagent consumption of DPA and WIZ probes are compared. The reagent consumption of the WIZ was calculated by using the information on the real consumption of reagents after a two weeks field deployment session. The low consumption permitted the design of a "plug-in" compact reagent container to allow an immediate field reagent and calibration solutions changeover, ensuring real field portability; it can contains up to 500 ml of solutions in several flexible bags.

TABLE I
Reagents consumption DPA vs. WIZ

Reagent	DPA consumption (µL)	WIZ consumption (µL)
R1 - Acid molybdate (PO_4)	180	60
R2 - Ascorbic acid (PO_4)	180	60
R1- Sulphanilamide (NO_2)	900	120
R2 - Napthylethylenediamine (NO_2)	900	120
R3- DTPA (NO_3)	900	225
R1 - OPA (NH_4)	1400	225
Cleaning (Dichlorocyanuric acid, DIC)	900	225

DPA probe WIZ probe

Analytical procedure

The nutrients in-situ probe is configured to measure automatically and unattended the nutrient parameters in sea water or fresh water using standard wet chemical methods [17,18]. During the sample intake, the sample is filtered through a 10µm metal filter screen and during cleaning cycles the filtering unit is flushed several times with pressurized sample water and compressed air to prevent any back clogging of the filter. The DPA or the WIZ analyzers provide the sampling frequency necessary to obtain the detailed distribution of the various analytes in the water column and the system will adjust itself autonomously to the specific detection limits. The general analytical sequence and the procedures are summarized in Table II.

TABLE II
ANALYTICAL PROCEDURES FOR NUTRIENTS

	PO_4-P [10,11]	NH_4-N [9,10]	(NO_3 + NO_2)-N [9,10]	NO_2-N [9,10]
Filtration	yes	yes	yes	yes
Sampling	90 sec	90 sec	90 sec	90 sec
Sample blank reading	---	yes	---	---
Injection of first reagent	Acid Molybdtate	OPA	DTPA	SAA
Sample blank reading	yes	---	yes	yes
UV reduction	---	---	5 min	---
Second reagent injection	Ascorbic acid	---	SAA	NED
Mixing	yes	yes	yes	yes
Third reagent injection	---	---	NED	---
Temperature conditioning	25 °C*	45 °C, 6 min.	---	---
Duration of analysis	5 min	12 min	12 min	5 min
Flow cell	20 mm	10 mm	20 mm	20 mm
Wavelength/ Detection method [9,10,11]	880 nm, Spectrophotometric	370/420nm Fluorometric	525 nm Spectrophotometric	525 nm Spectrophotometric
Range	5-500 µg/L	3-1000 µg/L	10-500 µg/L	2-200 µg/L
Wash cycle	50 sec	50 sec	50 sec	50 sec
Inlet line back wash	---	---	---	5 sec, DIC /acid

* if required under low ambient temperature, water sample temperature could be increased to 25 °C

The analytical methods presently in use for phosphates, nitrates, nitrates and ammonia is as follows:

- orthophosphate (PO_4-P), is measured using acid molybdate solution and ascorbic acid and the blue color of the phosphor-molybdenum complex formed is measured spectrophotometerically at 880 nm as described in [17,18,25].

- ammonia (NH_3-N), is determined fluorometrically (at 370-390 nm excitation and 420 nm emission), using orthopthaldialdehyde (OPA) as the fluorogenic agent [27] with sodium sulfite and sodium tetraborateborate as a buffer [27,28]. The method is highly selective and sensitive [27] and matrix effects are minimal [28].
- nitrate + nitrite (NO3+NO2)-N, using the nitrate UV photo reduction and subsequent determination of reaction products as nitrites [28,29]. During the sample prepossessing step, DTPA and TRIS buffer is added to the sample, the mixture is then subjected to UV irradiation in a UV digester. The digestion step facilitates photo-reduction of nitrates to nitrites [28]; the nitrites formed by the photo-reduction then react with sulfanilamide and naphtylethylendiamine in acid solution to form a pink colored azo dye.
- nitrite (NO_2-N), is measured spectrophotometrically using N-(1-naphtyl)ethylendiamine di-HCl (NED) and sulphanilamide (SAA). The nitrite in the sample is in a strongly acid medium (HNO_2) reacts with NED and SAA to form a pink colored azo compound measurable at 525 nm [17, 18].

III. RESULTS AND DISCUSSION

Instrument performance

The evaluation of performance characteristics is performed at the University of Natural Resources and Applied Life Sciences, Vienna (BOKU). Standard solutions with known ion concentration, as well as real river water are used for this evaluation. Performance characteristics, like linear working range, accuracy and reproducibility (stability) as well as limit of detection (LOD) and limit of quantification (LOQ) were evaluated thoroughly using approved validation methods and statistically based estimation and the results shown in Fig. 4.

The evaluation of repeatability and accuracy is performed through measurement of a mixed synthetic solution containing 20 – 40 µg/L each ion in the time of nearly one week (n= 40). The results of this experiment (Fig. 4) show that stable and accurate results can be achieved for phosphate, ammonium and nitrite. The recovery of mean concentration (n=40) is between 79 and 122% and the relative standard deviation between 3 and 6%. A concentration decrement for ammonium is observed at this experiment (Fig.4) and this is to explain with the non-stability of this ion under the given experimental conditions (open vessels, room temperature). This evaluation experiment is repeated for ammonium under controlled conditions and the results for recovery and repeatability are added (Fig. 4), for 14 consequently measurements.

LOD and LOQ are both evaluated by repeated measurement of a blank (10 times) and estimating the LOD/LOQ from the average concentration (C_{blank}) and its standard deviation (SD_{blank}), according to (1) and (2).

$$LOD = C_{blank} + 3 * SD_{blank} \quad (1)$$

$$LOQ = C_{blank} + 10 * SD_{blank} \quad (2)$$

This experiment is repeated four times and the average values for LOD / LOQ are shown in Fig 4. The LOD / LOQ estimated are comparable to them of the standard laboratory methods, which makes the µ-LFA technology very attractive for continuous water monitoring.

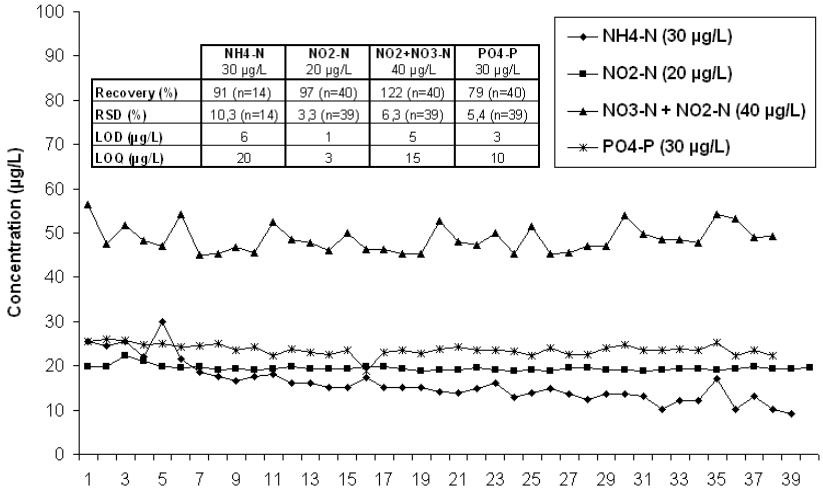
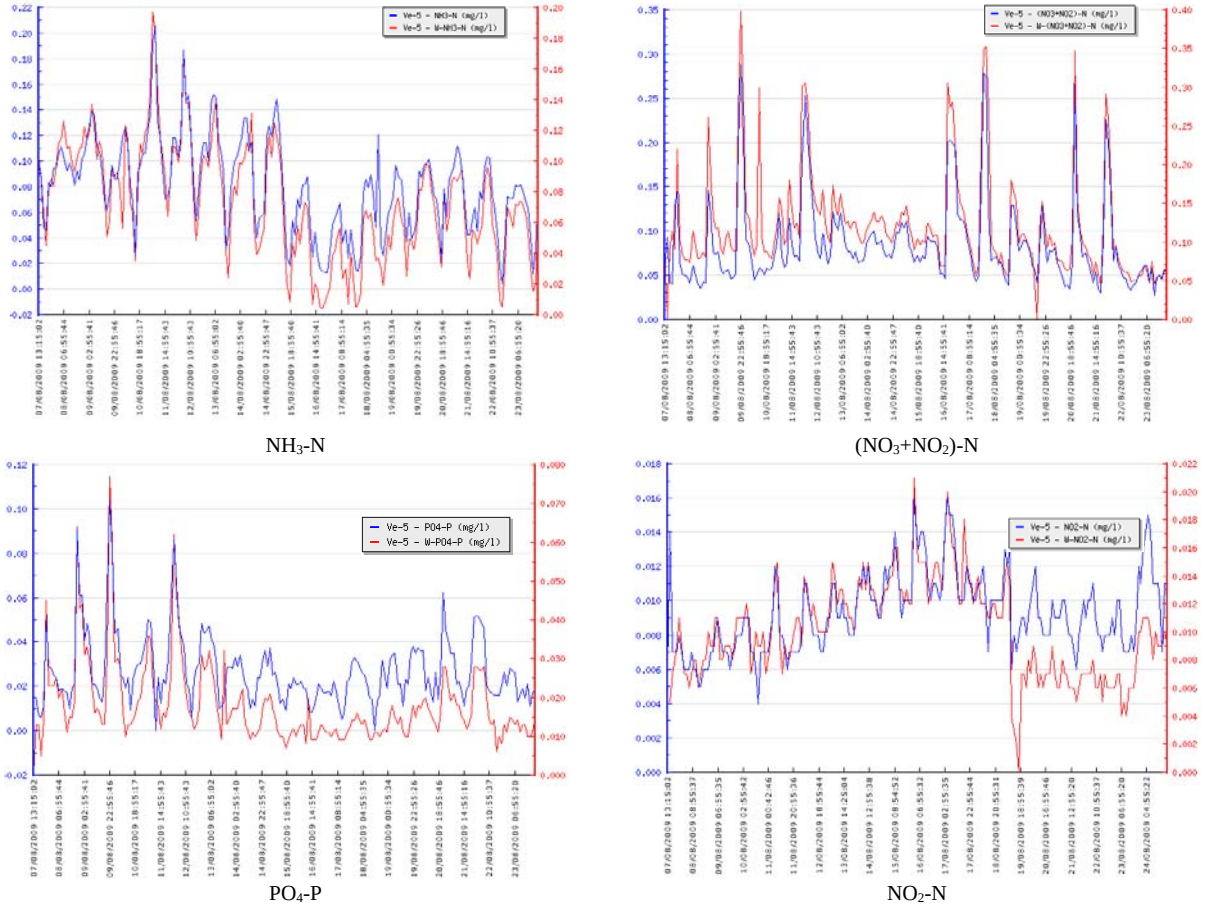


Fig.4 DPA repeatability and accuracy test results



NH_3-N (NO_3+NO_2)-N

PO_4-P NO_2-N

Fig.5 Data comparison between DPA and WIZ probe in VE-5 (Porto Marghera)

The deployment of both DPA and WIZ multiparametric probes together at Porto Marghera, Venice (Italy) between 7 – 25 August 2009 show agreeable results with a similar diurnal variations for all four parameters measured (NH_4-N, NO_3-N, NO_2-N and PO_4-P) and are shown in Fig. 5. Both probes show highly significant correlations for NH_4-N (R^2=0,89), NO_3-N (R^2=0,84), and PO_4-P (R^2=0,76); R^2 is lower for NO_2-N (R^2=0,45) but still records a positive correlation. The reagent change (for nitrites) on 19th Aug. seems to have caused a drop in nitrate concentration of 4-6 µg/L (and hence the lower correlation coefficient) but it is unrecognizable in the N-NOx values due to high concentration of nitrate in the samples.

Due to resuspension of anoxic sediments and fall of redox values, nitrates get transformed fast into nitrites and finally to ammonium; with small traces of remaining nitrites in the water column is an indication of this. Nitrogen speciation in the shallow water column is mainly influenced by lagoon dynamics influenced by change in hydrological and other physico-chemical factors, and also human influences may contribute to these changes (Fig.5). For example clam fishery done by mechanical raking of sediments in the vicinity of the station, causes resuspension of anoxic sediments and increased water temperature facilitates fast reaction rates and hydrodynmics influence distribution patterns.

However, results from both probes show that nutrient concentrations begin to deviate from the normal pattern after 10-12 days, probaly due to external bio-fouling effects of the probes, showing regular maintence is reqired in highly eutrophic environments. In contrast, the internal biofouling in the system is totally eliminated through regular activation of wash cycle with DIC, which cleans up the internal circuit inclusive of all tubing continuously.

IV. CONCLUSIONS

The evaluation of performance tests carried out at the University of Natural Resources and Applied Life Sciences, Vienna (BOKU) shows that µ-LFA technology provides a useful tool for accurate measurement of nutrients measurements. The probes

deployed at the lagoon possess reagent autonomy for 3-4 weeks as they carry sufficient quantity of nutrients and DI water to analyze four nutrient parameters continuously at 2h intervals. The on-going experiments at Venice Lagoon also show that both DPA and WIZ probes could be deployed for three weeks even in a highly eutrophic, turbid environment (high SS content) with large Macroalgal and phytoplankton biomass, with minimal maintenance.

In general both analyzers fulfill the requirements that are needed for in-situ continuous monitoring such as multiple parameter analysis, self calibration, analytical accuracy, high reliability, real-time data transmission, low maintenance costs, and resistance to biofouling but improvements are needed in this aspect.

The advance features of the WIZ and its benefits could be summarized as follows: the reduced reagent consumption (Table-1) and reduced DI water use (max. 1 liter per month), both these features lead to low waste production thus requiring less space for waste and total storage in the probe. The reduced power absorption (3W standby, 6 W analysis, max 1 A) requires less self-generated power on a buoy or platform, or longer autonomy when using a battery. Improved design and engineering has resulted in a compact body by merging two chamber model DPA into a single unit with reduced weight. Easily changeable reagent container and their reduced size require less refrigeration space on-board for storage; in case of installation on coastal buoys, less installation space is required for the probe and smaller and weight enables handling by a single person. Also true portability allows the use for short term deployments, without the integration need on a coastal buoy or platform, and availability of auxiliary bags during stand-alone deployments is an advantage for longer deployments. Finally all these improvements bring a significant final cost benefits for the user.

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REFERENCES

[1] R.A. Vollenweider, “Scientific fundamentals of the eutrophication of lakes and flowing waters with particular reference to nitrogen and phosphorous as factors in eutrophication,” Report on Water Management Research, Organisation for Economic Cooperation and Development, DAS/CSI/68.27, OECD-Paris, 194 pp., 1968.

[2] S.E. Jørgrnsen, H. Löffler, W. Rast and M. Straškraba, “Lake and Reservoir Management,” Developments in Water Science -54, Elsevier, Amsterdam, 2005.

[3] A. Gunatilaka, & J. Dreher, “Use of real-time data in environmental monitoring – Current practices,” Water Sci. Technol., Vol. 47 (2), pp.53-61, 2003.

[4] E. Mostert, “The European Water Framework Directive and water management research,” Phys. Chem. Earth, vol. 28, pp.523-527, 2003.

[5] À. Borja, “The European Water Framework Directive: A challenge for nearshore, coastal and continental shelf research,” Cont. Shelf Res., vol.25, pp.1768-1783, 2006.

[6] J.J.Hudson, W.D.Taylor, D.W. Schindler “Phosphate concentrations in lakes,” Nature, vol.406, pp.54-56, 2000.

[7] S.J. Guildford, and R.E. Hecky., “Total nitrogen, total phosphorous and nutrient limitation in lakes and oceans: Is there a common relationship?” Limnol. Oceanogr., vol.45, pp.1213-1223, 2000.

[8] L.R- Adornato, E.A. Kaltenbacher, D.R. Greenhow and R.H. Byrne, “High-resolutiuon in-situ analysis of nitrate and phosphate in the oligotrophic ocean,” Environ. Sci. Technol., vol. 41, pp.4045-4052, 2007.

[9] J.West, M.Becker, S. Tombrink and A. Manz, “Micro total analytical systems: latest achievements,” Ana. Chem., vol.80, pp.4403- 4419, 2008.

[10] W. Yao, “Determination of nanomolar concentrations of nitrite and nitrate in natural waters using long path length absorbance spectroscopy,” Environ. Sci. Technol., vol.32, pp.2646- 2649, 1998.

[11] M. Rawlinson, “NAS-2 E in-situ analyzer a device for the reliable measurement of nutrient concentrations in the aquatic environment,” In Proc. IEEE Conf. Oceans 2000, vol.2, pp.853- 857, 2000.

[12] A. Hanson. “A new in situ chemical analyzer for mapping coastal nutrient distribution in real time”, OCEANS 2000 MTS/IEEE Conference and Exhibition, Vol.3 pp.1975-1982. 2000. DOI: 10.1109/OCEANS.2000.882229.

[13] G. Griffiths,“Monitoring the deep – the challenge of detecting hidden pollutants,” J. Ocean Tech., vol.1, pp.36-56, 2006.

[14] N. Cardellicchio, M. Luzzana, “Messa a punto di un prototipo di un analizzatore colorimetrico programmabile per la determinazione automatica dei nutrienti in acque di mare,” *Atti del seminario Tecnico Scientifico del Progetto Strategico “Monitoraggio automatico dell’inquinamento marino nel Mezzogiorno”* , Lecce, Italy, 1993, pp137-158.

[15] F. Azzaro, E. Crisafi, G. Magazzù, F. Oliva and A. Puglisi, “Un nuovo fotometro automatico per la determinazione di nutrienti da boa oceanografica,” *Atti ”Workshop: il monitoraggio automatico dell’inquinamento marino“*, Taranto, Italy, 1994, pp.213-226.

[16] F. Azzaro and M. Galletta, “Automatic colorimetric analyzer prototype for high frequency measurement of nutrients in sea water,” *Mar. Chem.*, vol. 99, pp. 191-198, 2006.

[17] K. Grasshoff, “Methods of seawater analysis,” with contributions by A. Almgreen, R.Dawson, M.Erhardt, S.H. Fonselius, B.Josefsson, F.Koroleff and K. Kremling, Verlag Chemie, Weinheim, 1976.

[18 J.D.H Strickland and T.R. Parsons, “A practical handbook of seawater analysis,” Bulletin 167 (Second Ed.), Fisheries Research Board of Canada, Ottawa, 1972.

[19] P. Moscetta, US Patent 5411708 “Apparatus for the determination of analytes in liquid samples,” May 2 1995.

[20] G. Zappalà, G. Caruso, F. Azzaro and E. Crisafi, “Marine environment monitoring in coastal Sicilian water,” in Water Pollution VIII: modeling, monitoring and management, Vol. 95 of WIT transactions on ecology and the environment, by C. Brebbia and J. S. Antunes do Carmo, pp.337-346, WIT press 2006, ISBN 184564042X, 9781845640422

[21] E.J. Palmer-Felgate, H.P. Jarvie, R.J. Williams, R.J.G. Mortimer, M. Loewenthal and C. Neal, “Phosphorus dynamics and productivity in a sewage-impacted lowland chalk stream”, *J. Hydrol*., vol.351, pp.87-97. 2008.

[22] J. Sarrazin, P.M. Sarradin, A.G. Allais, D. Almeida, S. Bernagout, R. Battencourt et al., “MoMARETO: a cruise dedicated to the spatio-temporal dynamics and the adaptations of hydrothermal vent fauna on the mid-Atlantic ridge,” International Research, Vol.15 pp.24-33. 2006.
[23] P.M. Sarradin,J. Sarrazin, E. Sauter, B. Schillito, C. Waldmann, K. Olu et al., “EXOCET/D Publishable Final Activity Report – October 2007”, Extreme ecosystem studies in the deep ocean: Technological developments, 9 p. (http://www.ifremer.fr/exocetd/documents/results/Finalactivity.pdf; accessed on 18 Aug. 2009).
[24] R. Vuillemin,P.M.Sarradin, C. Le Gail, D. Birot and N. Le Bris,, “In –situ flow-analysers for the chemical characterization of deep-sea hydrothermal vent habitats” European Geosciences Union General Assembly, 24-29 April, Vienna 2005. (http://www.google.at/search?hl=de&source=hp&q=Exocet%2FD; accessed on 28 Aug. 2009). 9 p. (http://www.ifremer.fr/exocetd/documents/results/Finalactivity.pdf; accessed on 18 Aug. 2009).
[25] J.Murphy and J.P.Riley,“A modified single solution method for the determination of soluble phosphate in natural water,” Analytica Chim. Acta. vol.27, pp.31-36, 1962.
[26 R.Kérouel, A. Aminot, “Fluorometric determination of ammonia in sea and estuarine waters by direct segmented flow analysis,” *Mar. Chem.*, vol.57, pp.265-275. 1997.
[27] R.M. Holmes, A. Aminot, R. Kérouel, B. A. Hooker and B.J. Peterson, “A simple and precise method for measuring ammonium in marine and freshwater ecosystems,” *Can. Fish Aquat. Sci.* vol.56, pp.1801-1808. 1999.
[28] Y. Zhang and L. Wu, “Photochemical reduction of nitrate to nitrite in acqueous solution and its application to the determination of total nitrogen in water,” *Analyst*, 111, pp.767-789, 1986.
[29] M. Di Leo and L Nollet, “Handbook of water analysis,” Marcel. Dekker, Inc., p.209, 2000.
[30] D. Thouron, R. Vuillemin, X. Philippon, A. Lourenço, C. Provost, A. Cruzado et al., "An Autonomous Nutrient Analyzer for Oceanic Long-Term in Situ Biogeochemical Monitoring", - 2003, Analytical Chemistry, Vol. 75, 2601-2609.
[31] R. Vuillemin, D. Le Roux, P. Dorval, K. Bucas, J.P. Sudreau, M. Hamon et al., “CHEMINI: a new in situ CHEmical MINIaturised analyzer”, Deep Sea Research, Instruments and Methods, 2009, 56, pp 1391-1399.