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# APPLICABILITY OF THE SPECTROSCOPY IN THE ANALYSIS OF SCUBA DIVERS RESPIRATION

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**Abstract**

Scuba diving could increase free radicals production which acts as signaling molecules to induce adaptation against oxidative attack. The aim of the new research was to study the impact of scuba diving effects on multiple gas composition responses from the exhaled breath of professional divers. Ethylene, ammonia, and carbon dioxide concentrations from diver's respiration were measured before and after the immersion to 8 m depth in the Black Sea. The ethylene gas (for oxidative stress condition), the ammonia gas (for proteins degradation) and the carbon dioxide gas from the respiration of the six divers were evaluated by mean of the breath test using infrared laser absorption spectroscopy. The experimental results for each breath test of scuba divers will be presented and discussed.

*Key words:* divers, gases emission, respiration assessment, spectroscopy

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## 1. Introduction

The scope of the present study was to analyze with help of the photoacoustic spectroscopy, the health consequences of diving that could be reflected by the oxidative attack which can be produced by the demand for physical activity (followed by increased production of free radicals inducing lipid peroxidation -LP) that can take place in different contexts (Perovic et al., 2014).

Breath is one of the best biofluids in the gas phase that can be easily and noninvasively collected from an individual for monitoring his metabolic state. Collecting/analyzing breath samples is preferred to direct measurement of blood samples, because it is extremely simple, painless, non-invasive and cheap and contamination is easily avoided. In the frame of the present research, volatile organic compounds (VOCs) will be studied by means of IR spectroscopy (9-11 μm). Such spectroscopy, being very sensitive to biological molecules due to the fundamental absorption bands in infrared (IR) allows both VOC identification via its unique fingerprint and measurement of its concentration.

The LP reaction consists in the degradation of cellular membrane phospholipids and ends to the formation of low-weight hydrocarbons such as ethane, pentane, and ethylene. We marked the appearance of the ethylene by using a $CO_2$ laser-based spectroscopic system. Part of the ethylene formed as a by-product of the LP reaction and expressed to the lungs by the sanguine fluids are eliminated with the breath in very low concentrations (ppb or ppm levels). Choosing an adequately sensitive technique for detecting ethylene, it is achievable to determine the extension of the oxidative stress in a clear and rapid way by the mechanism of a breath test (BT) (Perovic et al., 2014; Puiu et al., 2007).

Another compound found in the scuba divers respiration can be ammonia ($NH_3$) that is given first by the emergence of urea in the liver and formed by all tissues throughout the metabolism of distinct compounds. A higher level of ammonia in the breath induces pathophysiologic changes in the human

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organism being an information that suggest specific anomalies that may be present if breath ammonia becomes exalted (Popa et al., 2015). Our body produces carbon dioxide ($CO_2$) as a by-product of metabolism and is evacuated via the lungs (Cherry et al., 2009; Hickey et al., 1983). The $CO_2$ levels in the arteries in specialized nerve centers in the brain are called central chemoreceptors. Our brains go through this message and trigger our respiratory muscles when the $CO_2$ level get to a specific point. In normal conditions, the lungs will give sufficient ventilation to allow the $CO_2$ level in the circulation system to remain constant. With this goal (photoacoustic spectroscopy application on health consequences of scuba diving), the motivation of the study presents an experiment for detecting any small variation of carbon dioxide, ammonia and ethylene concentrations in the exhaled breath by scuba divers before and after a prolonged record immersion, conducted at the NILPRP (National Institute for Laser Plasma and Radiation Physics) Research Institute, Laser in Life Sciences, Environment and Manufacturing Laboratory (http://llasem.inflpr.ro/).

The present manuscript reports a new research with reference to the impact of scuba diving effects on multiple gases composition from scuba divers respiration by means of a dedicated optical system using gas phase by rovibrational spectroscopy, providing a new looks on breath-based metabolomics: new medical routine applications oriented towards IR gas analyzers and optical method with photoacoustic for subjects state control. The experimental results for each breath test of scuba divers will be reported and discussed.

## 2. Method and materials

### *2.1. Experimental procedure*

In this experimental study, the applicability of photoacoustic spectroscopy in the analysis of scuba diver's respiration was investigated. Numerous spectroscopic instruments, such as Faraday laser magnetic resonance spectroscopy or photoacoustic spectroscopy, can be used for determining a fully area of molecules. All the spectroscopic techniques present qualitative and quantitative details by analyzing the light absorption in a substance. Infrared absorption spectroscopy is a common method to acquire the spectral signatures of molecules with high resolution and high sensitivity (Popa et al., 2015; Popa, 2016). In the last years, a higher attention was designated to the photoacoustic spectroscopy laser-based instrument for the reason that this is a method that may warrant a higher sensibility with respect to the traditional spectroscopic techniques. Our investigation implicated the evaluation of ethylene, ammonia, and carbon dioxide molecules using the $CO_2$ laser photoacoustic spectroscopy that is suggestivelly drawn in Fig. 1 and completely disputed in another research manuscripts (Dumitras et al., 2010; Dumitras et al., 2007; Petrus et al., 2017; Popa, 2016; Popa et al., 2018). In brief, the photoacoustic detection system, composed on the resonant photoacoustic detector cell (containing the gas mixture sample) and a tunable continuously wave $CO_2$ laser source as the source had been used. The sensing path is ensued by a gas mixture scheme designed for a suitable control of the gas molecules in the investigation. The gas handling system asures secure gas purity in the photoacoustic cavity and it can be used to pump out the cavity, to fix up the divers breath sample in the cavity, keeping the total and partial pressures of gas mixtures and also can realize many actions without making required any disconections (Dumitras et al., 2007; Petrus et al., 2017). To measure the exhaled complex mixture of gases, it is fundamental to customize the resonant cavity with a recognized gases mixture and to indicate the linear responses of the detector for low detection of ethylene, ammonia, and carbon dioxide, reported also in Dumitras et al. (2010); Dumitras et al. (2007), Petrus et al. (2017), Popa et al. (2015), Popa, (2016), Popa et al. (2018), and experimentally determined using commercially prepared, certified gas mixtures diluted in pure nitrogen.

The value of the acoustic signal in the photoacoustic spectroscopy measured by the microphones, normalized to the size of the $CO_2$ laser radiation power, is comparative to the molecular absorption coefficient of the analyzed gas sample at a $CO_2$ laser radiation wavelength used. The assessment of ethylene, ammonia and carbon dioxide were performed for specific $CO_2$ laser lines. For ethylene detection at 10.53 μm, for carbon dioxide at 9.53 μm and for ammonia at 9.21 μm (Dumitras et al., 2010; Dumitras et al., 2007; Petrus et al., 2017; Popa et al., 2015; Popa, 2016; Popa et al., 2018).

This absorption device has seen notable progress according to the vast number of applications for non-invasive test of gaseous molecules, mainly in the area of medicine (Schwarz et al., 2009; Smith et al., 2014; Shende et al., 2017), biology (Amelynck et al.,2003; Davis et al., 2005), and environmental pollution monitoring (Milligan et al., 2002; Yuan et al., 2017). The photoacoustic enginery that we use has already provided results in the fields of biology and medicine with the detection of trace gases both in vitro and in vivo (Dumitras et al., 2010; Dumitras et al., 2007; Popa et al., 2018).

The development of laser sources and advances in laser spectroscopic techniques have led to new approaches to the analysis of biomarkers in human respiration. The main advantage of diagnosis by breath analysis is the non-invasive nature of tests that can be applied quickly and easily. Prior to the introduction of respiratory tests into clinical practice, the origin of respiratory biomarkers should be identified if they are metabolic or caused by oral contamination. A procedure for identifying the origin of respiratory biomarkers was performed by Bratu (2018).

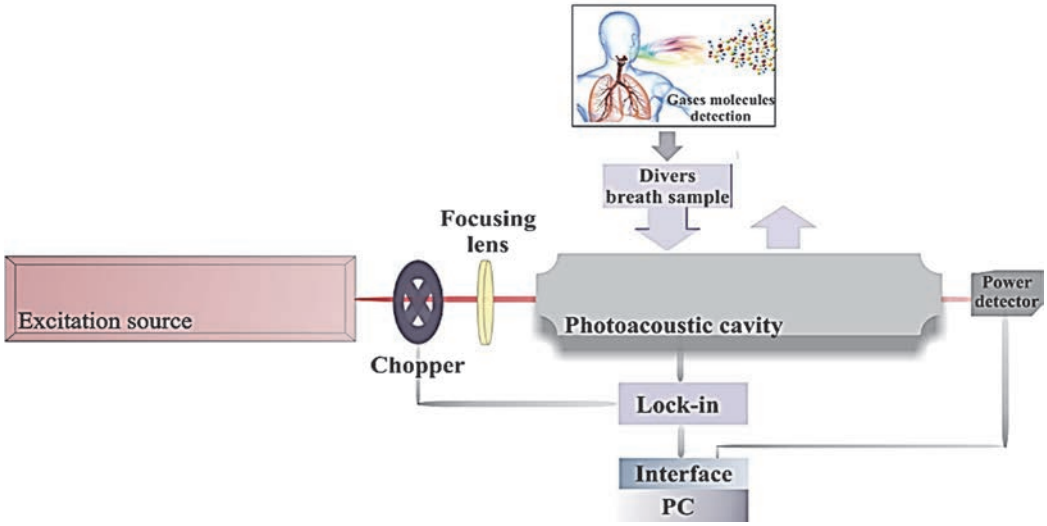



**Fig. 1.** The photoacoustic spectroscopy system for the analysis of scuba diver's respiration

Exhaled breath by mouth and nose before and after brushing with toothpaste / baking soda were evaluated in order to identify the important endogenous biomarkers without contaminant sources using laser photoacoustic spectroscopy. Several analytical uses of laser PA spectroscopy for the analysis of clinically relevant compounds in medical diagnosis have been presented in the literature. In particular, the ability of $CO_2$ laser-based PA systems to detect gaseous molecules with high sensitivity to sub-ppb levels has been proven.

Navas et al. (2012) presented in an invited review the advantages and disadvantages of photoacoustic spectroscopy for the analysis of human biomarkers in breath. Several analytical uses of PA spectroscopy for the analysis of breath of ethylene, nitrous oxide, ammonia and other clinically relevant compounds in medical diagnosis were analyzed. Eight years later, Dumitras et al. (2020) presents in a review an update of the state of the art photoacoustic spectroscopy technique for the analysis of clinical respiratory gases and describes their applications in cancer therapy, renal failure, diabetes, autism, schizophrenia and many others.

*2.2. Study protocol*

Six non-smokers professional divers (three males and three females) were recruited for this experimental investigation (35 ± 7.6 yr, BMI 22.52 ±4.2 kg/m$^2$). The professional divers were well-informed about the purpose and demands of the experimental study before giving their written consent to participate in this research (Table 1).

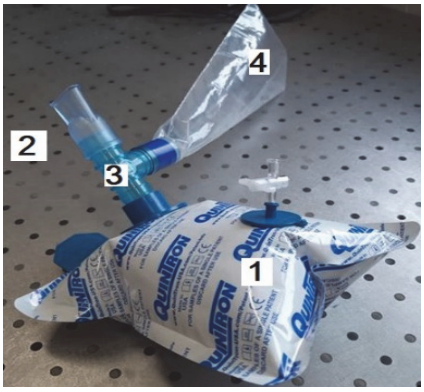
The subjects did not take any antioxidant dietary supplement or any routine medication for 1 month before the study and prior to the research, the divers were asked to keep away for at least 6 hours, before or at any time pending the test collection, alcohol and coffee, food or beverages. On the previous day to the investigations,were avoided onions, leeks, eggs, and garlic.For the baseline, we used the control values obtained previously by the LPAS experimental set-up (Popa et al., 2011; Popa et al., 2018). The control values are 0.006 ppm for ethylene, 0.25 ppm for ammonia (Popa et al., 2011) and 1.75 ppm for carbon dioxide (Popa et al., 2018).

Divers performed an immersion in the Black Sea to a depth of 8 meters for a total time of 40 minutes in which they breathed atmospheric air supplied from scuba tanks. Non-patented divers, Scuba Discovery, have a maximum diving limit of 10 meters. Due to the group of divers on which the research was done (not all patented) and the relief of the Black Sea in the area where the dives were made, 8 meters was the maximum possible depth. The analyses of the respiration were made from all divers' prior immersion as well as after diving.

**Table 1.** Scuba divers information

| *Subject* | *Gender* | *Age* | *Subject height (m)* | *Subject weight (kg)* | *BMI (kg/m$^2$)* | *Ponderal Index (kg/m$^3$)* |
|---|---|---|---|---|---|---|
| S1 | Female | 31 | 1.57 | 50.0 | 20.28 | 12.92 |
| S2 | Male | 49 | 1.85 | 77.0 | 22.50 | 12.16 |
| S3 | Female | 28 | 1.59 | 48.0 | 18.99 | 11.94 |
| S4 | Female | 30 | 1.57 | 53 | 21.50 | 13.70 |
| S5 | Male | 37 | 1.89 | 110 | 30.79 | 16.29 |
| S6 | Male | 35 | 1.85 | 72.0 | 21.04 | 11.37 |

The sampling procedure (Figs. 2a-b) was realized in agreement with prior researches using bags coated with aluminum, projeted to accumulate multiple samples from divers and keep a sample for a maximum of 6 h. The diver places the piece designed to be put in his mouth (Fig.2b), forming a fixed seal around it with the lips and a natural exhalation of breath is then given via the mouth. When a proper breath sample is collected, the diver turn off the natural exhalation. The bags with breath are delivered in the laboratory and the accumulation with breath samples are transferred from the bags into the measuring cell where we can detect the traces of multiple gases.Valuable information about oxidative stress status in humans can be obtained through exhaled ethylene monitoring based on a non-invasive BT (Petrus et al., 2017; Popa, 2016; Popa et al., 2018; Puiu et al., 2007).




(a)

(b)

**Fig. 2.**(a): 1- 0.75 L Aluminium-coated bags QuinTron, USA; 2- Mouthpiece; 3- Tee connector ; 4-0.40 L Discard bag. (b): Diver breath protocol collection

The breath composition can be continuously monitored or otherwise checked through the collection of single samples to be subsequently analyzed in the laboratory. Taking into account our practical application, we had to make the mixed expiratory collection method. In terms of the origin of the collected breath gases, there are three basic collection approaches: upper airway collection for NO test; this means that only deadspace gas is collected (it is only for the NO test); mixed expiratory collection; this means that total breath air, including dead space air and alveolar gas is collected (appropriate for tests of special gases and VOCs); alveolar collection; this means that pure alveolar gas is collected (for tests of other inorganic gases and VOCs). Because the mixed expiratory collection method is easy to perform in spontaneously breathing subjects requiring no additional equipment, it has been used by our practical application.

The breath samples were collected and stored in special aluminized bags (0.75-liter Quintron) in which the breath has to be exhaled without previous hyperventilation. The bags are provided by a one-way valve and a mechanism for discarding the bronchial air.

## 3. Results and discussion

### *3.1. Results*

In this research study, gases concentrations from the diver's respiration were assess before and after the immersion to 8 m depth in the Black Sea (Figs. 3-5). Non-patented divers, Scuba Discovery, have a maximum diving limit of 10 meters. Due to the group of divers on which the research was done (not all patented) and the relief of the Black Sea in the area where the dives were made, 8 meters was the maximum possible depth. To show the presence of oxidative damage we measured ethylene as a byproduct of LP and Fig. 3 shows the concentrations of breath ethylene for diver's respiration. Sampling was made from both divers prior immersion as well as after diving. The ethylene level for the first diver: S1 respiration before the immersion is 0.415 ppm than after the immersion where it is easily elevated (0.424 ppm); for the second diver: S2 exhaled breath before the immersion is 0.5 ppm than after the immersion (0.53 ppm); for the third diver: S3 respiration before the immersion the ethylene concentration is 0.476 ppm than after the immersion (0.453 ppm) when it is insignificantly reduced; for the fourth diver: S4 respiration before the immersion is 0.461 ppm than after the immersion (0.496 ppm); for the fifth diver: S5 respiration before the immersion is 0.5 ppm than before the immersion when it is insignificantly low (0.497 ppm); and for the sixth diver: S6 respiration, the concentration is the same before (0.5 ppm) and after the immersion (0.5 ppm). As an observation of our primary determination of interest, we see that the results given by all samples (from Fig. 3) show no significant increase or decrease in the exhaled breath ethylene, in particular, no measured value goes over the limits. The confounding factors such as age or sex showed no significant differences between the subjects.

Fig. 4 presents the concentrations of breath ammonia from the respiration of the six divers to verify if breath ammonia becomes elevated or not.In extension to breath ethylene, we measure also the breath ammonia to show or not the presence of pathophysiologic changes in the diver's organism, ammonia being a byproduct of amino acids (Brannelly et al., 2016). The breath samples were taken both for professional diver's prior immersion as well as after diving. The ammonia concentration for the S1 respiration before the immersion is 1.58 ppm than after the immersion where the concentration is decreased at 0.83 ppm; for the S2 respiration before the immersion, the ammonia concentration it is 1.78 ppm compared to the concentration after the immersion (1.32 ppm); for the S3 respiration before the immersion, the ammonia it is 2.5 ppm than after immersion (1.9 ppm); for the S4 respiration before the immersion we have a concentration of 1.9 ppm compared to the concentration after the immersion (0.91 ppm); for the S5 respiration before the immersion is 2.5 ppm compared to the ammonia concentration after the immersion where suddenly falls to 0.7 ppm; for the S6 respiration before the immersion it is 1.4 ppm than after the immersion where the concentration of ammonia decreases insignificantly at 1.3 ppm. Factors such as age or sex showed no significant differences between the subjects.

As an examination regarding the results given by all samples, from Fig. 4, we can observe that no increase in the exhaled breath of ammonia of professional divers were found after the immersion, and on the contrary, we find it a slightly low level after the immersion when we compared to the respiration of the scuba divers before the immersion.

Because the diver may not have been able to breathe quickly enough to eliminate the $CO_2$ that was building up in their bloodstream, in addition to ethylene and ammonia we measure also the $CO_2$ level for the diver's respiration. In this way, Fig. 5 presents the concentrations of $CO_2$ from the respiration of the six divers to verify if $CO_2$ level from the bloodstream (via lungs) remains relatively constant.

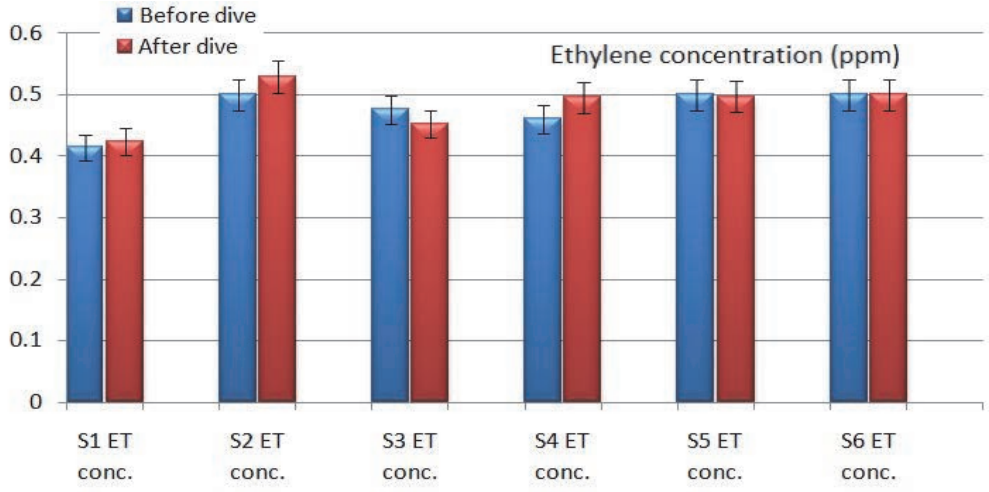



**Fig. 3.** Determination of ethylene from scuba divers respiration

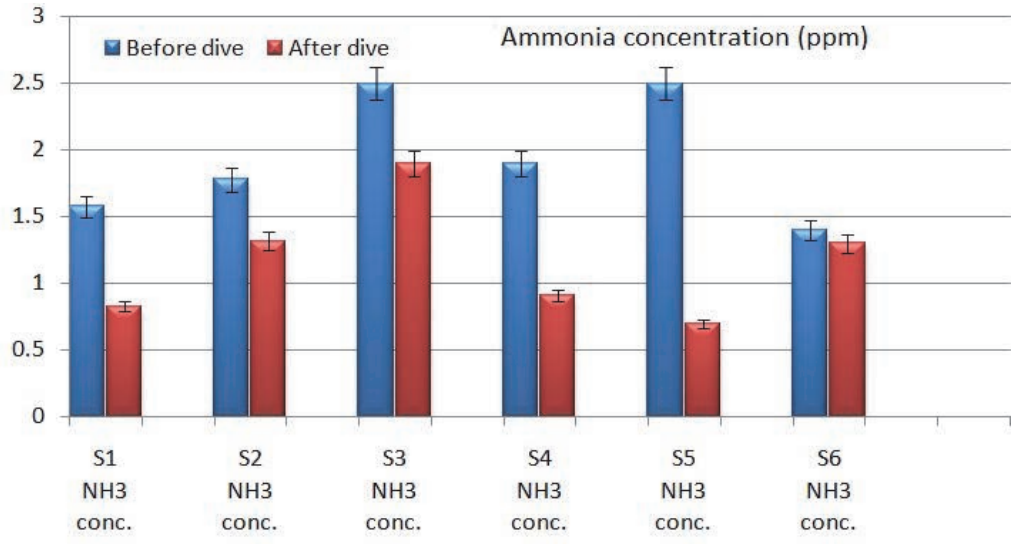



**Fig. 4**. Determination of ammonia from scuba divers respiration

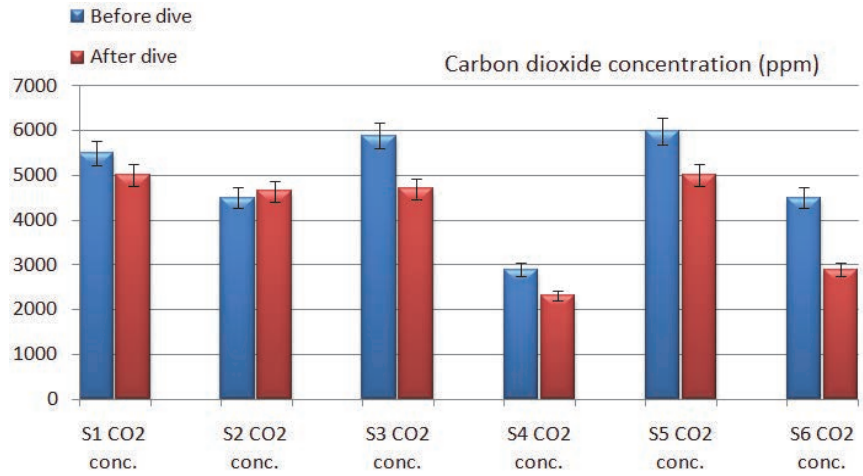



**Fig. 5.** Determination of carbon dioxide from scuba divers respiration

The $CO_2$ level for the S1 before the immersion has a value of 5500 ppm than after the immersion where the concentration is slightly low (5000 ppm); for S2 before the immersion is 4500 ppm than after the immersion (4650 ppm); for S3 before the immersion is 5890 ppm than after the immersion (4700 ppm); for S4 before the immersion is 2890 ppm than after the immersion (2310 ppm); for S5 before the immersion is 6000 ppm than after the immersion (5000 ppm); for S6 before the immersion is 4500 ppm than after the immersion (2890 ppm). Factors such as age or sex showed no significant differences between the volunteers.

As an analysis regarding the results given by all samples, from Fig. 5, we can observe that the $CO_2$ from scuba divers breath was found to be in a relatively lower concentration after the immersion than before the immersion to 8 m depth in the Black Sea.

### *3.2. Discussions*

From the results of this investigation, the ethylene breath divers were identified with no important differences after the immersion when we compared to the respiration of the divers before the immersion. The stress state of the divers was assessed by mean of the abovementioned BT for the physiological stress assessment. Sampling was made from both divers prior immersion as well as after diving. The results show no significant increase in the exhaled ethylene. Previous studies reported in the literature (Perovic et al., 2014; Puiu et al., 2007) presented no important increase in the exhaled ethylene of divers after the immersion when is compared to ethylene before immersion. Instead, a small increase of ethylene was observed during the dive. So, our results confirm previous determinations that oxidative stress was not meaningfully increased. As ethylene is originated as a secondary product of oxidative stress, ammonia is produced as a secondary product of amino acids (Popa et al., 2011; Popa et al., 2015; Popa et al., 2018). Higher breath ammonia determines pathophysiologic changes in the diver's organism being an evidence that disturbances may be present if breath ammonia becomes elevated. In addition to breath ethylene, we measure also the ammonia level for the diver's respiration and we report that the ammonia breath divers were identified as slightly low after the immersion when we compared to the respiration of the divers before the immersion.

Our body produces $CO_2$ as a by-product of metabolism and is evacuated via the lungs. Diving can significantly alter the body's ability to eliminate $CO_2$. The divers may not have been capable to respire fast enough to remove the $CO_2$ that was building up in their bloodstream.

Carbon dioxide is a toxic gas that is odorless, tasteless, and colorless and signifies the gaseous end result of the aerobic metabolism of oxygen. $CO_2$ is able to be dissolved in body tissues and easily spreads from cells to blood, where circulation transfer it to the lungs for the expulsion. Carbon dioxide may have express and detrimental effects if a diver accumulates an excessive amount of $CO_2$, and for this reason, scuba divers frequently verify their compressed air so they can guarantee the air is safe to inhale underwater (Cherry et al., 2009; Xiang et al., 2013; Benedek, 2018). Scuba tanks are filled by compressors that use special filters to dry and clean the air of harmful particles.

A previous study conducted at the Duke Center for Hyperbaric Medicine and Environmental Physiology found four variables that can influence arterial $pCO_2$ during immersed: depth, which corresponds to gas density; external breathing resistance; individual maximal oxygen consumption ($VO_2max$); and individual hypercapnic ventilatory response (Begin et al., 1976; Bleakley and Davison., 2010; Cherry et al., 2009; Ivascu et al., 2016; Roland et al., 2011; Srámek et al., 2000; Sureda et al., 2014; Xiaojuan and Guomin, 2014). The assessment of the subject's $CO_2$ level was measured with an end-tidal $CO_2$ meter, which measures the $CO_2$ level in the exhaled gas (Cherry et al., 2009). In addition to ethylene and ammonia, we measure also the $CO_2$ level

for the diver's respiration and we report that the $CO_2$ from breath was found to be in a relatively lower concentration after the immersion than before the immersion to 8 m depth in the Black Sea.

As a general conclusion, the data from this study encourage the premise that diving is possible, at least for professional scuba divers without physical and psychological disorders. In this study, factors such as age or sex showed no significant differences between the subjects.

## 4. Conclusions

The breath tests of six divers were analysed by infrared absorption spectroscopy in order to evaluate ethylene, ammonia and carbon dioxide compounds. Infrared absorption spectroscopy was selected as a sufficiently sensitive method for revealing with high specificity and sensitivity in the multiple gases exhaled by the six scuba divers subjects. The physiological assessment was concluded without any evident clinical consequence of the stress condition in the six divers, probably because of their high practicing level. The reported data results are not sufficient for a statistic study, but it is certainly evident that the time evolution of exhaled gases does not seem random.

Based on a non invasive enginery, fixed in biological materials and simple to estimate; we gather that infrared absorption spectroscopy test of breath ethylene, ammonia and carbon dioxide in diver respiration appeared to be a viable tool for monitoring real-time concentrations of multiple gases in diver's breath. Being able to extract a signal from flowing breath samples, provided the advantage of collecting large amounts of data in a short period of time. Furthermore, the sensitivity and accuracy were highly supportive of the results found in previous literature.

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