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## APPLICATION OF THE ACOUSTICS USING LASER TECHNOLOGY IN THE EVALUATION OF THE BIOLOGICAL SAMPLES RESPIRATION

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**Abstract:** Cadmium (Cd) is a heavy metal (HM) that presents a great environmental problem due to its high toxicity to all living organisms. The aim of our study was to monitor the ammonia (NH<sub>3</sub>), ethylene (C<sub>2</sub>H<sub>4</sub>), ethanol (C<sub>2</sub>H<sub>5</sub>OH) and carbon dioxide (CO<sub>2</sub>) biomolecules emissions in *Triticum aestivum* plantlets growth with or without Cd, using photoacoustic spectroscopy in order to suggest new markers that may contribute to a better understanding of Cd phytotoxicity. Laser photoacoustic spectroscopy results show that seeds germinated with Cd, determine a slowly increase of NH<sub>3</sub> vapors in the respiration of plantlets, a decrease of C<sub>2</sub>H<sub>4</sub>, and a greater increase of C<sub>2</sub>H<sub>5</sub>OH and CO<sub>2</sub> vapors in the respiration of *Triticum aestivum*.

**Keywords:** photoacoustic spectroscopy, acoustics, biomolecules, heavy metal

### 1. INTRODUCTION

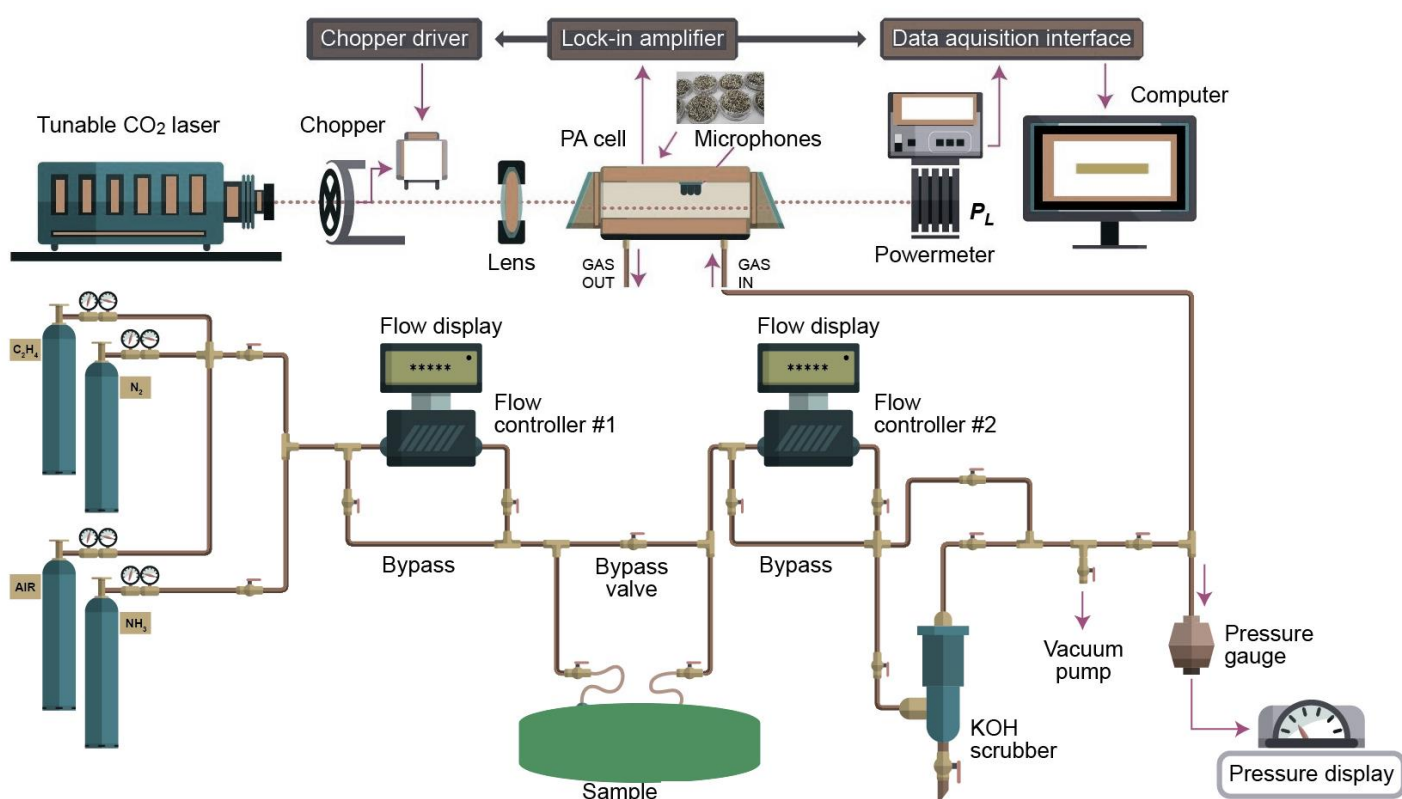
Heavy metals are naturally occurring metallic elements with a higher density than water, higher than 5 g/cm<sup>3</sup> [1]. A part of these metals (Fe, Mn, Zn, Co, Cu and Ni) are macro- and micronutrients necessary in metabolic processes related to plant growth and so, can be removed, to some extent, from soil, through phytoextraction [1,2]. Other, such as Cd, Cr, Pb and Hg don't have any known biological function and additionally can become toxic to the plants at higher concentrations [2]. There are several sources of heavy metals in the environment which originate either from natural processes (volcanic eruptions, rock weathering, and soil forming processes) or anthropogenic processes.

However, the latter is the most alarming. Due to recent human activities, such as mining, industrial processing and use, agricultural practices, etc. it was reported an increase of environmental contamination which in the end means a higher toxicity to humans [3-5].

The purpose of this research was to investigate the effect of Cd on *Triticum aestivum* by monitoring the different volatile organic compounds- VOCs ( $\text{NH}_3$ ,  $\text{C}_2\text{H}_4$ ,  $\text{C}_2\text{H}_5\text{OH}$ , and  $\text{CO}_2$ ) emitted by the plantlets under stress conditions.

## 2. TECHNICAL REQUIREMENTS

The  $\text{CO}_2$  laser photoacoustic spectroscopy set-up used for the determination of the  $\text{NH}_3$ ,  $\text{C}_2\text{H}_4$ ,  $\text{C}_2\text{H}_5\text{OH}$  and  $\text{CO}_2$  molecules is presented in Fig. 1 and extensively described in previous papers [6-10].



**Figure 1:** Photoacoustic  $\text{CO}_2$  laser photoacoustic spectroscopy set-up used for the determination of the  $\text{NH}_3$ ,  $\text{C}_2\text{H}_4$ ,  $\text{C}_2\text{H}_5\text{OH}$  and  $\text{CO}_2$  molecules

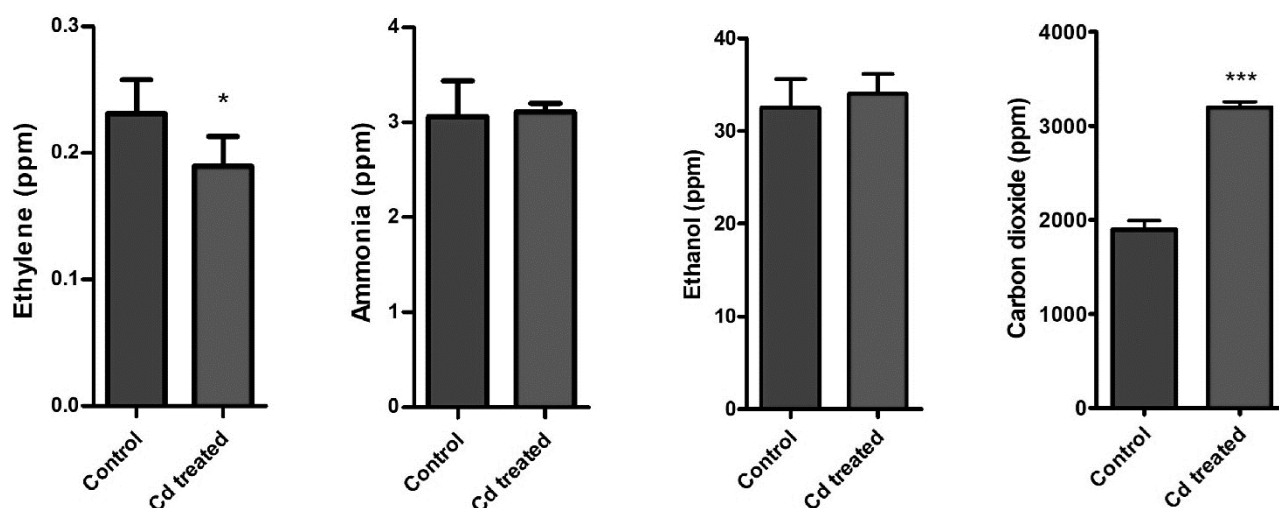
The laser photoacoustic spectroscopy detection set-up consists of a tunable continuously wave (CW) frequency stabilized  $\text{CO}_2$  laser, emitting radiation in the 9.2–10.8  $\mu\text{m}$  region on different vibrational-rotational lines, with a maximum power of 6.5 W, a lens system, a chopper, a photoacoustic cell, a powermeter, a lock-in amplifier, an acquisition board and a computer for data acquisition and processing. The acoustic detection chain is accompanied by a complex gas handling system built for a proper manipulation of the gases molecules under study, from the gas bottle to the photoacoustic cell.

The calibration measurements performed using commercially prepared, certified gas mixtures were reported previously [6-11].

The absorption coefficients of NH<sub>3</sub>, C<sub>2</sub>H<sub>4</sub>, C<sub>2</sub>H<sub>5</sub>OH and CO<sub>2</sub> at different CO<sub>2</sub> laser wavelengths were precisely measured previously [10, 11] and the CO<sub>2</sub> laser was kept tuned at the 10.53 μm where C<sub>2</sub>H<sub>4</sub> exhibit a strong peak, corresponding to an absorption coefficient of 30.4 cm<sup>-1</sup>atm<sup>-1</sup> and at 9.22 μm, where the NH<sub>3</sub> absorption coefficient has the maximum value of 57 cm<sup>-1</sup>atm<sup>-1</sup>, at the 9.258 μm where C<sub>2</sub>H<sub>5</sub>OH exhibit a strong peak, corresponding to an absorption coefficient of 4.08 cm<sup>-1</sup>atm<sup>-1</sup> and at the 9.533 μm where CO<sub>2</sub> exhibit a strong peak, corresponding to an absorption coefficient of 0.00301 cm<sup>-1</sup>atm<sup>-1</sup>.

Ammonia, C<sub>2</sub>H<sub>4</sub>, C<sub>2</sub>H<sub>5</sub>OH and CO<sub>2</sub> molecules generated by the samples were measured at 80 h from the germination of the *Triticum aestivum* seeds in water or in the presence of Cd.

Figure 2 shows the level of the VOCs emitted by *Triticum aestivum* seeds after 80 h of germination with or without Cd.



**Figure 2.** NH<sub>3</sub>, C<sub>2</sub>H<sub>4</sub>, C<sub>2</sub>H<sub>5</sub>OH and CO<sub>2</sub> production for control or Cd germinated common wheat seeds. \*p < 0.05, \*\*\*p < 0.001; Unpaired t-test with Welch's correction.

For control plants, the concentrations of the VOCs found in the respiration of the plantlets were about 0.231 ± 0.013 ppm for C<sub>2</sub>H<sub>4</sub>, 3.063 ± 0.19 ppm for NH<sub>3</sub>, 32.50 ± 1.56 ppm for C<sub>2</sub>H<sub>5</sub>OH and 1898 ± 47.10 ppm for CO<sub>2</sub>.

The effect of Cd intake on *Triticum aestivum* seeds growth was detected through changes in the concentrations of the gases emitted.

The ethylene decreased from 0.231 ppm in control samples to about 0.19 ± 0.012 ppm for Cd treated ones. It appears that Cd acts as stress to plantlets and affects the plant physiology.

The NH<sub>3</sub> molecules concentration in the respiration of the plantlets treated with Cd is similar with the one found in control ones, i.e. 3.11 ± 0.045 ppm.

Similar results to control were found for C<sub>2</sub>H<sub>5</sub>OH molecules, which concentration is about 34 ± 1.08 ppm. However, we found a significant increase in the concentration of CO<sub>2</sub>, which reached about 3197 ± 30.43 ppm.

### 3. CONCLUSIONS

In the present research we analyzed the NH<sub>3</sub>, C<sub>2</sub>H<sub>4</sub>, C<sub>2</sub>H<sub>5</sub>OH and CO<sub>2</sub> gases molecules using laser photoacoustic spectroscopy at seeds germinated with Cd and we compared the results with the signal respiration of seeds (plantlets) germinated only with distilled water.

Cadmium treatments does not affect NH<sub>3</sub> and C<sub>2</sub>H<sub>5</sub>OH from in the respiration of plants, while we find a decrease of C<sub>2</sub>H<sub>4</sub> and a high increase of CO<sub>2</sub> vapors in the respiration of *Triticum aestivum*.

Using laser photoacoustic spectroscopy, we tried to see if we can find to suggest new and easier methods to assess markers that may contribute to a better understanding of Cd effect. The use of related gases elements in the respiration of plant tissue for seeds treated with Cd is acceptable in theory; metabolic modifications appear in plantlets treated with Cd that inevitably lead to the generation of exceptional metabolites. These elements are transferred through diffusion and the metabolites will then be released into the respiration of plantlets as molecule components of each biological sample.

Based on our findings, we can say that the laser photoacoustic spectroscopy technique could be an easy way to identify biological samples treated with Cd.

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