Optical absorption spectra of germinated seedlings from seeds exposed to vehicle polluting gases

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Abstract

High pollution rates in large cities have had a negative impact on human, animal and plant health. In the case of plants, several techniques have been used to analyze the physical and physiological parameters of their seedlings and seed germination process, under different environmental conditions. This study proposed the evaluation of seedlings from corn, lentils and bean seeds, exposed to polluting gases (CO, CO₂) through a container system of a vehicle engine, during different times (0, 20, 40, 60 and 80 min). The seeds, after the exposition to the pollutant gases, were planted, and the optical absorption spectra of each seedling were obtained by photoacoustic spectroscopy. The absorption spectra were obtained in a wavelength range from 250 to 750 nm. The obtained spectra demonstrated significant statistical differences in the wavelength range from 290 to 300 nm (corresponding to optical absorption mainly by flavonoids) and also from 650 to 680 nm (corresponding to optical absorption of chlorophylls). In addition, changes in physiological parameters, during the seed germination process, were evaluated.

Keywords Photoacoustic spectroscopy · Polluting gases · Air pollution · Seeds · Germination

1 Introduction

Environmental air pollution is one of the world's problems that has grown in recent years, causing approximately 7 Mio. deaths worldwide. According to WHO reports in 2018; 9 out of 10 people breathe air that contains high levels of contaminants [1]. Among the main sources of pollutants in the air are those produced by mankind due to the combustion of fuels in industry, heating, motor vehicles, etc., Fuels and oils are essential for the operation of motor vehicles; using both contributes to emissions of pollutants [2]. Many of these fuels and oils produce high emissions of nitrogen oxide (NO), volatile organic compounds (VOCs), ozone (O₃), fine particles (PM10 and PM2.5), carbon dioxide (CO₂) and carbon monoxide (CO) [3, 4]. Internal combustion engines use fuels that contain carbon,

for example gasoline, kerosene, oil, propane and coal [5]; in these engines, an incomplete combustion process produces CO at concentrations varying from 300 to 1200 and 1500 to 4000 ppm for diesel and gasoline engines, respectively; these high CO concentrations are due to the low presence of oxygen in the combustion of fossil fuels. CO is considered as one of the most important pollutants in the air [6–9].

The effects of polluting gases, emitted by thousands of vehicles every day in large cities, have been investigated by measuring the impact that they generate on animals [10] and vegetation [2, 11]. The literature reports the effects produced by the emission of motor vehicles on physiological behavior in plants, in the short and long terms, of different varieties of species and functional types, such as trees, shrubs and plants, exposed to CO and CO₂

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gas emissions produced by motor vehicle traffic [3, 11–13]. Some of these studies were conducted by evaluating, in an uncontrolled environment, seeds and trees growing near high-traffic roads in largest cities, with high pollution index [4, 11, 14–20].

On the other hand, few investigations deal with systems that replicate the conditions of pollutants produced on roads, which examine the direct impacts of vehicle engines on plants, and analyze separately the effects of responsible pollutants [3, 17, 21, 21, 22]. It has found that in nature the harmful effects may vary depending on the type of pollutants and the adaptation of the species to the polluted environment [3]. Different spectroscopic techniques, such as Raman, IR radiometry, thermal lens spectrometry and photoacoustic spectroscopy (PAS), have been used to obtain characteristic spectra in homogeneous and non-homogeneous materials [23]. Among these techniques, the photoacoustic spectroscopy stands out for its advantage over other spectroscopic techniques since in PAS most of the samples do not require a previous treatment, with chemical reactive, to be analyzed. Due to this advantage, PAS has allowed the characterization of different solid materials, powders, liquids, gases, biological samples and medical [24] and recently it has become a valuable tool in food science [25, 26].

PAS has been used to evaluate a wide variety of seeds, grains and seedlings providing valuable information on their physical properties [27–30]. Also PAS technique allows a nondestructive analysis from the optical absorption spectra of different samples, including optically opaque samples [31, 32]. PAS has allowed to obtain the optical absorption spectra of many types of solid or semi-solid samples including crystalline materials, powders, amorphous, gel, etc. In the present investigation, the effect of the polluting gases (CO and CO₂), emitted by a vehicle engine, on seeds of different crops, was evaluated. Studied seeds were treated before sowing, with the aim of analyzing the optical absorption spectra of their seedlings, in a wavelength range from 250 to 750 nm, and the physiological quality in the germination process.

2 Materials and methods

2.1 Biological material

In the present study, the used maize seeds (*Zea mays* L.) were grown during the spring–summer 2017 agricultural cycle, while the commercial lentil (*Lens culinaris*) and beans (*Phaseolus vulgaris* L.) were grown during the spring–summer 2018 agricultural cycle in the State of Mexico, Mexico. All seeds were selected and classified according to their weight, size and color.

2.2 Gas exposure system

For the exposure of samples to the polluting gases, a system was designed to contain these gases, which consists of a hermetically sealed container, 102 L capacity with dimensions 78 cm × 52 cm × 36 cm. Two mobile PVC grids were placed at the container ends, one grid in the left side, used for the entry of the polluting gases emitted by a vehicle engine with the following characteristics: 2003 model, 6 cylinders, gasoline engine without catalysts, high mileage lubricant type, recorded traveled distance: 170,000 km and the vehicle's exhaust is connected to a 100 cm long pipe so as not to expose the gas-containing system as well as the vehicle's engine to high pressures and temperatures. The right side of the container, with the other grid, is the gases outlet, considered so that the gas flows and does not saturate or causes high pressure within the system (Fig. 1). Also, a portable CO sensor (model: GCO-2008) was placed in the outlet grid, as an additional record to the internal sensors of the gas container system, to compare the data of both records.

For the automated measurement recording system, a program was designed in the Arduino free license software and its circuit was built with the integration of the sensors in real time. The used sensor models were: DHT11 (temperature and humidity), MG-811 (CO₂) and MQ-9 (CO), compatible with the microcontroller (Mega Arduino model) and connected to a computer for data recording. The circuit was placed inside the container, at the front (without obstructing the Petri dishes with the seed samples).

2.2.1 Pre-treatment exposure in vehicle exhaust system

The experiment was done with the experimental design of randomized complete blocks, with six repetitions for corn and bean seeds and four repetitions for lentil seeds, and the experimental unit for each repetition was 20, 20 and 40 seeds, respectively; the seeds were homogenized by size and subsequently placed inside of sterilized Petri dishes, 9 cm in diameter and 1.5 cm deep. Petri dishes without cover were distributed within the container with polluting gas, according to the experimental design.

The exposure times were 20, 40, 60 and 80 min for the different treatments applied to the seeds placed inside of the polluted gases container, emitted by the vehicle's engine. To maintain the CO and CO₂ levels at 2000 ppm, without reducing and not saturating the container with gases, the following procedure was performed: the engine remained on during 5 min, after this time the engine was off, because the cold start causes greater gas production due to the start driving cycle [9].

Fig. 1 Exhaust gas collection system

2.2.2 Germination

The pre-treated seeds were placed on filter paper inside sterilized Petri dish (9 cm in diameter and 1.5 cm deep) for the germination process. The established control was carried out without treatment of polluting gases. Petri dishes were placed according to the experimental design in boxes with white LED lighting programmed for an exposure time of 10 h per day, during the period of 10, 6 and 12 days for maize, lentil and bean, respectively. Irrigation was constant every third day in the morning, with a 5 ml supply of distilled water for each treatment. Germination was monitored daily and calculated the germination percentage (the germination percentage of the treatments was calculated considering the total number of normal seedlings among the total seeds, and the emergency rate index is calculated by daily counts of the number of seedlings emerged per day between the number of days after the seeds have been sown). At the end of the test, fresh and dry seedling weights (FW and DW) were measured. DW was obtained prior to washing and drying them, washed with distilled water and sodium hypochlorite (10 ml per treatment), in the case of removing pathogen residues in some of the seeds, then dried in the oven (model: Riossa E-51) for 12 h, at a constant temperature of 40 °C for 72 h. The seedlings of each variety (maize, lentil and bean) were crushed in a mortar to obtain powders thereof and be measured

by photoacoustic spectroscopy. For each measurement, the sample was removed and the PA cell cleaned using a paper towel.

2.3 PAS experimental setup

The PA spectra of seedling powders, of the seeds treated with CO and CO₂, were obtained by using the PAS technique in direct configuration in a wavelength ranging from 250 to 750 nm. Figure 2 shows the PAS experimental configuration, whose excitation source is a Xenon lamp with 700 W power, a monochromator at 1 nm step, with a resolution of ± 5 nm for sampling in wavelength. The monochromatic light beam is modulated by a mechanical chopper, at 17 Hz frequency; an optical fiber is used to guide the modulated light beam to the PA cell that inside contains the sample. The PA cell is hermetically sealed by using two quartz windows and silicone grease as a seal, as shown in Fig. 2. The absorbed light energy generates a modulated heating in the sample that causes periodic heat flow in the adjacent air and generates pressure fluctuations in the air contained within the PA cell. Finally, gas expansion and contraction variations give rise to acoustic waves, which are detected by means of an electret microphone connected to a lock-in amplifier to obtain the PA signal amplitude and phase; these data are acquired and recorded by using a computer.

Fig. 2 PAS experimental setup

3 Results and discussion

Figure 3 shows optical absorption spectra of seedlings samples of maize (a), lentil (b) and bean (c) treated pre-sowing by pollution gases at different exposure times (20, 40, 60 and 80 min). The spectra of these samples have different absorption bands, the first one related to flavonoids (around 295 nm) [33], the second band related to carotenoids (400 nm–500 nm) and the third one associated with chlorophyll (650 nm–700 nm) [34]. In PA spectroscopy, and also in the case of commercial UV–Vis spectroscopy, the samples are excited by a light source, where a wavelength scan is performed. For UV–Vis, the intensity of the transmitted light, through the sample, is sensed by an optical sensor which allows to obtain the optical absorption spectrum; by other hand, the absorbed light also provokes electronic transitions in molecular orbitals into the sample, these electronic excitations decay, through radiation-less transitions, and then, modulated heat is produced in the sample causing a periodic heat flow in the adjacent air and then pressure fluctuations in the air contained within the PA cell.

The PA spectra of maize seedlings samples are presented in Fig. 3a; the maximum value of the amplitude PA signal (0.37 mV) is observed at 300 nm wavelength for T_3 treatment (60 min), and the minimum amplitude (0.325 mV) for seedlings without treatment (control). Also it is observed that at 675 nm wavelength the treatment T_3 presents the maximum value of PA signal amplitude (0.224 mV) and the minimum amplitude value (0.149 mV) for T_2 (40 min). Thus, the amplitude level of the PA signal increased and decreased 31.76 and 14.1%, respectively,

when is compared with respect to the amplitude of the PA signal obtained from the seedlings without treatment (control). The PA signal of the seedlings coming from the treatments T_1 and T_2 showed a tendency of behavior similar to the control seedlings in the wavelength range of 300–525 nm. However, for the subsequent range, the level of the PA signal (525–750 nm) of these seedlings presented differences in the spectrum.

Figure 3b shows the PA spectra of lentil seedlings samples where the control samples showed the lowest amplitude level at absorption band of 275–320 nm, with respect to the seedlings from the other treatments applied. Note that the maximum absorption peak at 295 nm (0.55 mV) was for T_1 . In the absorption band corresponding to chlorophyll (650–700 nm), the amplitude of the PA signal for seedlings control and treatment T_4 (80 min) showed a similar behavior. The maximum absorption peak was presented by treatment T_2 (40 min), with an increase of 42.85% with respect to the amplitude of the signal from the control seedlings.

Regarding the bean seedlings (Fig. 3c), the highest amplitude of PA signal was found for treatment T_1 (20 min) in the wavelength range 275–320 nm, with an increase of 66% compared with control seedlings. The lowest amplitude of the PA signal was found for the treatment T_2 (40 min) with a decrease of 36.36%. At the peak of absorption corresponding to chlorophyll (675 nm), the control and T_4 (80 min) seedlings show the highest amplitude level of PA signal. The treatments T_1 , T_2 and T_3 had a decrease in the level of signal amplitude, with the highest decrease in T_1 (20 min). In the results of the investigation, the PA spectra of the seedlings obtained by means of PAS

Fig. 3 Photoacoustic spectrum of seedlings of: **a** maize, **b** lentil and **c** bean treated with pollutant gases emitted from vehicle at different exposure times (0, 20, 40, 60 and 80 min)

were analyzed, indicating that the level of the PA signal depends on the type of seeds, exposure time to polluting gases and the characteristics of the vehicle's engine.

In general, it is possible to say that seedlings from treated seed with CO and CO₂ gas pollution, emitted by the vehicle engine, produce changes in the level of PA signal at the characteristic absorption peaks of flavonoids (295 nm) and chlorophylls (675–700 nm). These changes may be associated with a stimulation such as those reported by some applied physical methods [33–36] as pre-sowing treatments which tend to cause positive, negative and null stimulation effects, evidenced in the literature with the evaluation of different quality variables, such as physiology; e.g., carotenoids. The carotenoids level has been qualitatively evaluated by PAS. Some authors have reported changes in the level of PA signal in the absorption band corresponding to carotenoids, in seedlings of

maize seeds, indicating increase or decrease in the level of signal amplitude according to the maize seed treatment based on applying laser light pre-sowing [37].

In this research, positive, negative and null effects were also found, according to the PA signal obtained for each treatment and seed type. Being PAS a useful technique to detected changes in seedlings due to gases pollution exposure, the PAS technique has been reported to evaluate change in bean seedlings due to the application of different concentrations of herbicides, obtaining a decrease in the PA spectrum in herbicide-treated bean leaves compared to controls. These authors indicated that the decrease in the spectrum intensity could be caused by the inhibition of photosynthetic evolution of oxygen or by a production of heat of the leaves due to the inhibition of photosynthesis by herbicides [32–34]. In this sense, the exposed seeds to polluting gases could affect their

photosynthetic evolution, since there were changes in the PA signal level in the flavonoids peak (295 nm) and chlorophyll band (around 675 nm) [38].

As shown in Fig. 4, there is a tendency to increase, decrease and maintain a value close to the control seedlings at the wavelength of both 295 nm and 675 nm, corresponding to flavonoids and chlorophyll, respectively, depending on the seed species and the exposure times of polluting gases. Being the bean seedlings the ones that showed the greatest PA signal increase, at 20 min of exposure time, and the less affected were the maize seedlings (Fig. 4a). At 675 nm wavelength, the greatest increase corresponds to treated maize seedlings at 60 min, and the largest decrease in bean seedlings treated at 20 min (Fig. 4b). Lentil and bean seeds show lower stimulating effect compared to maize seed, this could be due to their high flavonoid content and its defensive compounds, and it allows it to adapt to possible stress conditions [38–42]. Various research reports that high concentrations of some pollutants present in gases can be harmful to plants [17, 43]. The results obtained showed that the level of the PA signal depends on the type of sample and the exposure time to the polluting gases. Other authors have reported that the emission of polluting gases reduces productivity [16, 44]. Also in Fig. 4a, b, in the error bars of the treatments, it is observed that for the wavelengths of 295 and 675 nm that were compared; it can be seen that the sampling error increases when the PA signal has a higher intensity and a minimum error when the PA signal strength is lower.

Table 1 shows the physiological results obtained during the corn, lentil and bean seeds germination, when exposed pre-sowing to polluting gases at different times 0, 20, 40, 60 and 80 min. Statistically significant differences

were found in germination percentage (GP), emergency velocity (EV), fresh weight (FW) and dry weight (DW) in the case of maize seed; in this seed, the GP parameter had significant statistical differences on days 2, 4 and 6. There was a seed stimulation by the treatment with polluting gases on the corn seeds. There was a highest increase in germination percentage in treatment T_4 , increasing with respect to the control, 48%, 8% and 4%, at 2, 4 and 6 days, respectively. The stimulation of GP was higher on the second day of sowing established. At 2, 4 and 6 days, the emergency velocity increased by 18%, 16% and 12%, respectively. On the contrary, the measured fresh and dry weights decreased with the increase in the applied treatment time, the maximum decrease was for treatment T_4 , with a decrease of 6% and 7%, for FW and DW, respectively, when compared with the control sample (T_0).

In the case of the lentil and bean seed, there were no significant statistical differences in the evaluated variables (GP, EV, FW and DW), but there was a behavioral trend. In the case of bean seeds on days 2 and 4, there was a tendency to increase the germination percentage, but at days 3 and 4, it tended to decrease. Similar results were found for emergency velocity. But in the case of fresh and dry weight, a slight tendency to increase was found, when comparing T_4 with control sample (T_0), increasing 6% and 10% the FW and DW parameters, respectively. In the case of lentil seed, on days 3, 4, 5 and 6, both for the germination percentage and emergency velocity showed a tendency to increase (between 6 and 9%), and fresh and dry weights parameters showed a behavior tending to increase 5% and 4%, respectively.

The behavior of GP is shown in Fig. 5, column (a1), (b1) and (c1) for maize, lentil and bean seeds, respectively. The GP value for maize seeds Fig. 5a1, within the period

Fig. 4 Photoacoustic signal of maize, lentil and bean seedlings samples at **a** 295 nm and **b** 675 nm

Table 1 Comparison of average values of germination variables of treated seeds at different exposition times to pollution gases

Days		2	3	4	5	6	2	3	4	5	6	12	12
T(min)		GP2	GP3	GP4	GP5	GP6	EV2	EV3	EV4	EV5	EV6	FW (gr)	DW (gr)
<i>Maize</i>													
T ₀	0	27.5	50	73.3	82.5	85	2.75	6.08	9.75	13.05	15.9	16	8.19
T ₁	20	26.7	50	70.8	80.8	80.8	2.66	6	9.54	12.77	15.5	15.63	8.08
T ₂	40	34.2	55	77.5	85.8	87.5	3.41	7.08	11	14.39	17.3	14.47	7.24
T ₃	60	34.2	50	79.2	88.3	89.2	3.41	6.75	10.7	14.24	17.2	14.58	7.29
T ₄	80	40.8	50.8	79.2	88.3	89.2	4.08	7.47	11.4	14.96	17.9	14.75	7.31
LSD		8.67	5.75	5.98	6.26	5.85	0.86	1.02	1.13	1.26	1.37	0.6	0.24
Mean		32.7	51.2	76	85.2	86.3	3.26	6.67	10.5	13.88	16.8	15.08	7.62
S		0.017*	0.32	0.02*	0.06	0.03*	0.01*	0.03*	0.01*	0.0074*	0.005*	0.0001**	0.0001**
V.C		22	9.33	6.53	6.11	5.63	22	12.8	8.96	7.55	6.79	3.35	2.71
R ²		0.56	0.55	0.63	0.6	0.64	0.56	0.6	0.6	0.62	0.62	0.73	0.86
<i>Lentil</i>													
T ₀	0	48.5	74	88	90	90	12.1	24.5	35.5	44.45	52	4.73	2.32
T ₁	20	43.5	76.5	92	94	94	10.9	23.6	35.1	44.52	52.4	4.8575	2.31
T ₂	40	50	84.5	94.5	96.5	96.5	12.5	26.6	38.4	45.73	56.1	4.78	2.34
T ₃	60	46	78	93.5	95.5	95.5	11.5	24.5	36.2	46.85	53.7	4.82	2.37
T ₄	80	48	81	94	96	96	12	25.5	37.3	46.85	54.9	5.01	2.43
LSD		15.7	11.2	7.8	7.89	7.89	3.93	5.44	5.71	6.04	6.38	0.28	0.08
Mean		47.2	78.8	92.4	94.4	94.4	11.8	24.3	36.5	45.92	53.8	4.84	2.35
S		0.9	0.33	0.41	0.41	0.41	0.9	0.79	0.71	0.65	0.61	0.32	0.05
V.C		21.7	9.21	5.54	5.42	5.42	21.7	14.2	10.2	8.54	7.69	3.79	2.41
R ²		0.4	0.48	0.32	0.32	0.32	0.4	0.42	0.44	0.45	0.45	0.73	0.53
<i>Bean</i>													
T ₀	0	7.5	17.5	35.8	55.8	77.5	0.75	1.91	3.7	5.94	8.52	21.61	6.58
T ₁	20	9.16	19.2	20	40	78.3	0.91	2.19	3.19	4.79	7.4	20.98	6.84
T ₂	40	10.8	20.8	21.7	41.7	77.5	1.08	2.47	3.55	5.22	7.8	22.04	7.43
T ₃	60	10	20	20.8	40.8	79.2	1	2.33	3.37	5	7.64	21.15	7.05
T ₄	80	12.5	22.5	22.5	42.5	82.5	1.25	2.75	3.87	5.57	8.32	22.4	7.25
LSD		3.57	3.57	9.3	9.3	5.4	0.35	0.59	0.84	1.14	1.27	1.59	0.63
Mean		10	20	24.2	44.2	79	1	2.33	3.54	5.3	7.94	21.63	7.03
S		0.08	0.08	0.01	0.01	0.31	0.08	0.08	0.49	0.27	0.35	0.34	0.08
V.C		29.7	14.9	32.2	17.6	5.7	29.7	21.2	19.8	17.96	13.4	6.13	7.51
R ²		0.6	0.6	0.56	0.56	0.58	0.6	0.6	0.27	0.26	0.26	0.45	0.53

T seed exposure time to contaminants, GP germination percentage, EV emergency velocity, FW fresh weight, DW dry weight, LSD low significant difference, VC variation coefficient, R² determination coefficient, S significance (***)

of 5–6 days, the T₁ treatment (20 min) was lower than the seed without exposure to polluting gases (control), while in others, treatments GP had better performance. Figure 5b1 shows the GP value of the lentil seeds, where it is observed that from the third day of germination, all treatments show an improvement when compared to the control. At the end of the germination process, the T₂ treatment shows the best GP value and the lowest value for control.

In Fig. 5c1, the bean seed treatments show that during the period of 3–6 days it was observed lower GP values for seeds exposed to the polluting gases, when compared to

the control sample, while at the sixth day, the treatments show an improvement in their GP behavior compared to the control. At the end of the germination process, the T₄ treatment has the highest GP value. Figure 5a2 shows that the treatment T₄ (80 min) has the best EV performance compared to the control and T₁ shows the worst performance. For lentil seeds, all the treatments show better EV performance than the control (Fig. 5b2), whereas for bean seed, the T₄ treatment shows the best EV performance compared with the other treatments and control (Fig. 5c2).

Fresh weight (FW) and dry weight (DW) values of germinated maize seeds are shown in Fig. 5a3, which show

Fig. 5 Physiological quality parameters of maize (a), lentil (b) and bean (c) at different pollutant exposure times (0, 20, 40, 60 and 80 min): (a1), (b1), (c1) germination percentage, (a2), (b2), (c2) emergency speed and (a3), (b3), (c3) fresh and dry weight

a trend in their behavior, highlighting that the control sample (without exposure of contaminating gases) shows higher FW and DW values than the seeds exposed to contaminants. On the other hand, the T_2 treatment (40 min) has the lowest FW and DW values. For the lentil seeds (Fig. 5b3), it is observed that the lowest FW and DW values correspond to the control and T_2 treatments, respectively, while T_4 has the best performance for both. For the bean seeds (Fig. 5c3), T_2 has the highest DW value and the control has the lowest; finally, in the FW measurements, the T_4 treatment has the highest value and T_2 the lowest value.

Another important factor observed during the germination process is that at a longer exposure time no fungi were found during the germination of maize and bean seedlings compared to the control where some seeds were infected. The effects of exhaust emissions from motor vehicles on the seed germination process vary according to the degree and time of exposure to the pollution gases and

the seed type. The wide distribution of flavonoids in lentil and bean seeds suggests their fundamental importance and prophylactically protection of the seedlings against pathogens [45].

The changes observed in the seed germinating could be due to the tolerance of each specie to pollutants that are absorbed by the different treatments and the accumulation of other pollutants from the engine exhaust of the used vehicle: e.g., hydrocarbons, lead, metal powder, soot particles, etc., that have to be considered an important additional factor responsible in the process of seed germination and seedling growth [17, 18, 44].

High concentrations of CO_2 , compared with the literature reports, have allowed to detect how it influences the physiological functions of the development of crops and seeds, it has been found that it reduces the germination of some rice seeds, the production of abnormal seedlings and the appearance of dead seeds are indicators of a

physiological deterioration presented in the seed, strongly indicating that a high level of CO₂ induced irreversible damage to the quality of the seed. CO₂ exposure at levels of 720 ppm reduces germination of the produced seed, while exposure to 610 and 720 ppm reduces seed vigor [46]. In the case of some legumes such as chickpea seeds, some authors suggest that the increase in atmospheric CO₂ could have an adverse impact on viability, germination and cause a nutritional imbalance [47]; in the case of beans, it has been reported that by increasing CO₂ concentration indices do not influence the seed composition, the emergence or vigor of the produced seedlings [48].

Another important factor, together with the polluting gases that affect the seed germination process, is the temperature [49], in the present research, the average temperature, during the exposure to the polluting gases, increased ~3 °C from initial temperature (27 °C), and this increment in the air temperature is associated with the increase in CO₂, CO and other polluting gases that could have substantially influence on the seed development and thus reducing their quality. This research shows that the results were stimulating and inhibitory depending on the seed and treatment.

4 Conclusions

The photoacoustic spectroscopy (PAS) technique is a useful tool that provides reliable information on optical properties in natural pigments of biological samples (seedling powder) such as carotenoids (with optical absorption band ranging from 400 to 500 nm) in the case of maize, flavonoids (with an optical absorption peak around 295 nm) and chlorophylls (with optical absorption band ranging from 650 to 700 nm). The characterized samples (seedling powders) were obtained from the germinated seeds, with previous treatment of different exposure times of polluting gases. The greatest changes in absorption spectra were obtained with respect to the control seed (without gas exposure), and the lentil seed has the lowest signal values in its optical absorption spectrum.

The physiological parameters during seed germination show a similar behavior shown in PAS. The effects of pollutants varied depending on the seed treatment, finding that the most sensitive seed, exposed to polluting gases, is the maize seed and the least affected was the lentil seed. Changes in GP and EV showed a tendency to improve in the most treatments exposed to polluting gases. The FW and DW measurements found the poor viability of the seeds exposed to pollutants finding a decrease in maize and differences that varied with the several exposure times for lentil bean seeds. In the case of maize and bean seeds, less pathogen infection was found for the longer exposure

time treatment ($T_4 = 80$ min) compared to the control of both seeds; finally, in the case of lentil seed, these effects were not observed.

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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