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A low-cost versatile system for continuous real-time respiratory activity measurement as a tool in environmental research

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ABSTRACT

Low-cost sensors available on the market allow reliable and straightforward research of model organism responses to changes in environmental conditions caused by xenobiotics or natural ecological processes. The purpose of this work was to develop a low-cost Arduino-based respiratory activity measuring system employing CO_2 sensors (ResTox) applicable for various research and risk assessment purposes, providing a stable platform and support for further development and extension of current capabilities. The presented system comprises hardware and accompanying software solutions. The instrument employs multiple digital CO_2 measuring sensors based on non-dispersive infrared (NDIR) technology, a waterproof temperature sensor, and a phototransistor light sensor. The cross-platform ResTox software support enables simple control of the measuring device and sensor output data storage during the experiments. Due to its uncomplicated and compact design, the developed system represents a versatile solution for experiments in both field and laboratory conditions and with animal and plant organisms.

1. Introduction

Respiration is a common physiological characteristic of all known life forms - from a micro to a macro scale. Respiratory rates of metabolically active organisms are objectively quantifiable, reflecting the magnitude of living processes essential for the survival of cells and organisms in terms of chemical products - particularly CO_2 [1]. When environmental conditions change, organisms express a range of physiological responses that result in mortality, reduced/enhanced reproductive success, growth rate, and activities of specific metabolic enzymes [2–6]. The variability of environmental conditions results in altered metabolic activity reflected in CO_2 production/uptake dynamics. Commonly employed techniques in measuring CO_2 production can be divided into direct (e.g., infra-red gas analysis, gas chromatography) and indirect (e.g., alkali and soda-lime trapping) methods [7] which vary in their sensitivity and accuracy. Assessment of respiration activity has also been used to monitor xenobiotics-induced stress in various organisms: soil microorganisms [8] , invertebrates [9,10] , vertebrates [11–13] and plants [14,15]. These researches highlight that changes in the CO_2 production rate of test organisms could be an early indicator of stress caused by exposure.

Numerous applications appear in different research areas, with a growing number of accessible and adaptable microcontrollers and sensors available on the market. Due to their low cost, simple handling, and accompanying documentation, these devices open up a possibility for users to develop customized sensor system solutions [16–21]; , requiring some fundamental expertise in electronics. Sensors based on non-dispersive infrared (NDIR) technology consist of a gas sampling chamber, an IR light source, and a detector on opposite sides and, as such, represent an application of infrared spectroscopy [22]. These devices operate on the principle of absorbance spectroscopy, calculating the concentration of the target gas (e.g., CO_2, N_2O, NO_x, SO_2, CH_4) in the test chamber based on the amount of the IR light absorbed by the detector, using the Beer-Lambert Law [23].

Although CO_2 uptake/emission dynamics is a valuable endpoint in ecological and ecotoxicological research, it is still rarely studied. In addition to the relatively high cost of CO_2 measuring devices, this type of research is limited by the lack of devices adapted to the specific requirements of the experiments. To overcome these challenges, this study aimed to test the usability of low-cost sensors in continuous monitoring of experimental organisms' respiratory activity exposed to xenobiotics and to develop a robust sensor-system framework that can be used as a

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starting point for the development of sensor-system solutions adjusted to the needs of different study designs. The chosen sensors provided high-quality data and were incorporated into a respiratory activity measuring system (ResTox). This paper comprises a detailed description of the ResTox sensor system (hardware and software solution) and demonstrates its performance through results obtained in experiments involving plant (duckweed) and animal (earthworms) organisms commonly used in ecotoxicological studies.

2. Materials and methods

2.1. Hardware components

An Arduino platform-based respiratory activity measuring system (ResTox) (Fig. 1a, Supplementary Material S1) comprising sensors for measuring parameters relevant for the analysis of biological activity was developed. Arduino MEGA (AM; Arduino Italy, https://www.arduino.cc/) was chosen as the central control unit of ResTox, being a mediator between the user and hardware elements of the developed instrument. With extended capabilities compared to smaller microcontrollers (number of available digital and analog pins, working memory), the AM board allows simultaneous management of multiple electronic devices and thus enables the realization of complex projects.

Sensors used for the construction of the ResTox system were selected based on the criterion of price-to-quality ratio among devices available on the market. Namely, one of the most important steps in the development of own low-cost sensor systems (i.e. DIY-projects) is a thorough research of the market and selection of mutually compatible components and sensors suitable for the desired application. For measuring the concentration of CO_2 in test chambers, the developed system employs digital CO_2 measuring sensors (SEN0220, MH-Z16 Intelligent Infrared Gas Module, Zhengzhou Winsen Electronics Technology CO., Ltd.) based on non-dispersive infrared (NDIR) technology, characterized by a wide effective range up to 50000 mg kg^{-1} CO_2 as well as high sensitivity, accuracy of ± 50 mg kg^{-1} + 5% reading value, stability (around 3% during the maximum recommended interval between two consecutive calibration procedures of 6 months), low power consumption and compatibility with various microcontrollers. The temperature is measured using a waterproof temperature sensor (DS18B20, Maxim Integrated Products, Sunnyvale, CA), which, due to its characteristics, is suitable for a variety of applications. Since the device is intended for use in various experimental setups, including those involving plant organisms, the ResTox system is equipped with a phototransistor light sensor (Shenzhen Haiwang sensor).

In addition to the sensors mentioned above, the system also contains a 12 V, 12 W vacuum pump (Ningbo Forever Electronic Appliance Co., Ltd.) of particular importance when tests are conducted in closed chambers and removal of air saturated with CO_2 is required.

During the measurements, CO_2 concentration data for each experimental chamber is displayed on a 20x4 character LCD screen providing the user with real-time readings updated every second.

The current version of ResTox comprises nine CO_2 sensors, among which eight are designated as experimental (Exp1-8) and are placed in test chambers. Simultaneously, the ninth serves as a reference sensor (Ref), providing an output used in noise reduction and correction procedures. The current setup is extensible for many sensors wired to the microcontroller, with 18 CO_2 sensors per AM board (Arduino MEGA RX pin limitations when running the SoftwareSerial Library necessary for communication with CO_2 sensors). Since the loads' total power requirement exceeds the maximum current available from the AM board, the CO_2 sensors are connected to an external 5 V power supply (Fig. 1b).

The AM board, the vacuum pump and the electronic connections shown in the wiring diagram (Fig. 1b) are enclosed in the main protective case of the proposed system. To facilitate handling, storage and customization of the ResTox system to various study designs, male USB type A connectors were soldered to each CO_2 probe. In turn, the required number of CO_2 probes can be easily connected to the system through the female USB connectors found at the rear of the central protective case (Supplementary Material S1). On the other hand, the temperature and light intensity sensor probes are nondetachable. The LCD screen, the LED indicators (Power, Error, Pump activity) and the On/Off switch are found on the front of the central protective case of the proposed system (Fig. 1a, Supplementary Material S1).

2.1.1. Calibration of CO_2 sensors

Calibration of the sensors had been performed before each experiment, according to instructions in the sensor documentation and EPA protocol [24], by exposing the sensors to stable environments of zero (400 mg kg^{-1}) and spanning (2000 mg kg^{-1}) concentrations of CO_2, for 20 min, respectively. Gas standards were used for the two-point calibration procedure (prepared by a Linde plc distributor: GTG plin d.o.o., Karlovac, Croatia).

2.1.2. Validation of CO_2 sensor measurements

Validation of gas sensor measurements was carried out by exposing sensors to gas standards of 500, 800, 1200, 1500, 1800, and 2000 mg kg^{-1} CO_2. All sensors were placed in a closed exposure chamber and

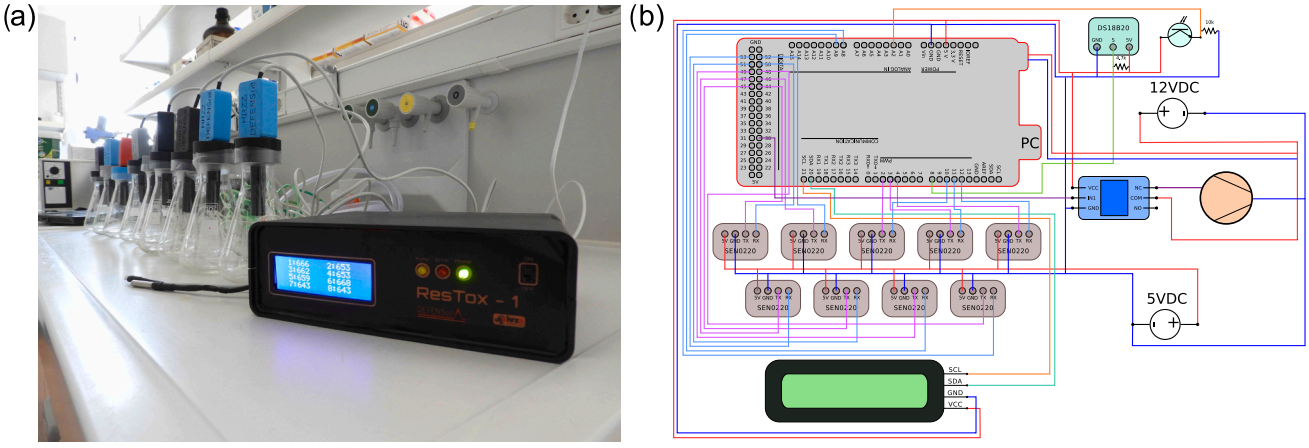

Fig. 1. The developed sensor system for respiratory activity measurement (ResTox) (a) and wiring diagram (b). The proposed system comprises an Arduino Mega microcontroller (shown in b)), nine NDIR CO_2 sensors (attached to Erlenmeyer flasks in a) and denoted as SEN0220 in b)), a temperature sensor (denoted as DS18B20 in b)), a light intensity sensor (phototransistor in b)), a vacuum pump (orange vacuum pump symbol attached to a relay in b)) and a LED screen (bottom left of b)). The microcontroller board and the vacuum pump are powered by 12 V (DC), while the CO_2 sensors require a 5 V (DC) power supply.

tested simultaneously, and sensor readings were recorded every second for 20 min. This process was repeated three times. Fitting sensor readings to a linear model (sensor readings vs. expected values) evaluated the accuracy of sensor measurements, and the readings' error was determined by calculating Root Mean Square Error (RMSE).

The applicability of the proposed system for assessing soil respiration was evaluated through comparative measurements with the Solvita soil respiration test system (Woods End Laboratories Inc., Mt Vernon ME, USA). Detailed description of the experimental setup is found in Supplementary Material S6.

2.1.3. Casing and adapters

Appropriate casings were designed to protect electronic parts of CO_2 sensors, as well as adapters and sensor holders for different test vessels (e.g., Erlenmeyer flask, Falcon tubes). Sensor cases and other accessories were designed in FreeCAD (version 0.17, https://www.freecadweb.org/) and produced using an Ultimaker 2 + 3D printer (Ultimaker B.V., Netherlands) fed with PLA material filament. Technical drawings of 3D printed components can be found in Supplementary Material S2.

2.2. Software

2.2.1. Arduino code

Operational code (Supplementary Material S3) for the AM microcontroller was written and uploaded using Arduino Integrated Development Environment (IDE) software (Arduino Italy, https://www.arduino.cc/). The uploaded program allows access to sensor output data, display of real-time data, and communication with the computer the board is connected to, in the sense of sensor output data transfer and reception of commands for pump activity control.

Furthermore, the AM board is programmed to execute sensor corrections during the measurement period, consisting of ambient noise reduction and a sensor error subtraction. In the first step, in the *setup()* stage of system boot, sensor errors are determined according to Equations (1) and (2):

$$c_{av} = \frac{\sum_{i=0}^{8} c_{Si}}{N_S} \tag{1}$$

where c_{av} is an average concentration of CO_2 at the beginning of measurement (considered the actual ambient CO_2 concentration), c_{Si} represents the CO_2 concentration output of each sensor (0–8, i.e., Ref + Exp1-8) and N_s the number of sensors used (in this study 9) and

$$e = c - c_{av} \tag{2}$$

where e represents the calculated error of each sensor (e_{Ref} denoting the error of the reference sensor and e_S representing the error of the experimental sensors), c stands for the CO_2 concentration output of each sensor (c_{Ref} denoting CO_2 concentration measured by the reference sensor and c_S standing for CO_2 concentration measured by the experimental sensors), and c_{av} is the previously calculated average ambient CO_2 concentration. Variables c_{av} and e (e_{Ref} and e_S for the reference and each experimental sensor, respectively) are stored as constants and are used in further calculations.

The following calculation sequence, performed in the *loop()* section of the Arduino code, is repeated every second during the measurement. In each timeframe, sensor output correction is carried out according to Equations (3) and (4):

$$\mathrm{n} = \mathrm{c_{Ref}} - \mathrm{e_{Ref}} - \mathrm{c_{av}} \tag{3}$$

$$c_{S\_corr} = c_S - e_S - n \tag{4}$$

where n stands for noise calculated in each timeframe, c_{Ref} and e_{Ref} represent the current output of the reference sensor and its previously calculated error, and c_{av} is the average ambient CO_2 concentration calculated according to Equation (1). Each sensor output (c_S) is corrected by subtraction of constant sensor error (e_S) and current noise (n), yielding the corrected value of CO_2 concentration c_{S_corr} for each sensor in each timeframe expressed in mg kg^{-1}.

To gain a better insight into the presented algorithm's performance, the AM board is programmed to send as much information as possible through serial communication - including raw sensor outputs, sensor errors, calculated noise values, and corrected sensor values. Additionally, the AM board also reads and records outputs of the temperature sensor and light intensity sensor, whose values are passed to the computer together with CO_2 data.

Apart from managing sensor data, the AM board also allows control of pump activity at user-specified intervals and duration by activating and deactivating a relay connected to it in response to commands sent by the computer through serial communication.

2.2.2. Software support

Software support accompanying the ResTox instrument was written in Kivy [25], an open-source Python library intended to develop cross-platform applications running on Linux, Windows, OS X, Android, iOS, and Raspberry Pi. The ResTox software support (Fig. 2) was created as an interface that enables simple interaction between the user and the instrument. Functions of the ResTox application are data logging, pump activation/deactivation, and real-time data display. Sensor outputs are saved at a user-defined location on the computer's hard drive connected to the ResTox instrument, for the specific time and with a frequency defined by the user. Furthermore, the user has full control over the frequency of vacuum pump activation and pump activity duration. Settings regarding data logging and pump behavior are defined in the SETUP segment of the application. Data received through serial communication with the AM board are saved in two files: .csv output for the user containing corrected CO_2 sensor values and light and temperature data and .rtox system log data containing all data received from the AM board.

The lower (MEASUREMENT) part of the GUI displays real-time sensor outputs. Information provided here includes the time remaining to the end of the experiment, time remaining to pump activation, real-time CO_2 sensor readings, current temperature, and state of light intensity registered by the photosensor, represented by a light bulb image dynamically changing based on light sensor outputs (on vs. off). Also, the application allows a preview of recorded CO_2, temperature, and light intensity data.

2.3. Experiments

The ResTox system's usability for ecotoxicological research was tested within experiments involving commonly used model organisms, representatives of both plant and animal organisms used in ecotoxicological tests.

The conducted experiments included nine CO_2 concentration measuring sensors – a reference and eight experimental sensors. The reference sensor was placed in a test vessel without experimental organisms.

CO_2 uptake/production rate was calculated as described in Peršić et al. [15]. Briefly, CO_2 data obtained were transformed by linearization, and uptake/emission rates (i.e., slope coefficient of the logistic model) were determined by linear regression of log-transformed CO_2 measurement data and time. Molar fractions of CO_2 (in units of mg kg^{-1}) were converted to volumetric mass density (in mg m^{-3}) using the ideal gas law. Plant surface area was measured using open source software for image processing and analysis, ImageJ 1.51j8 [26], from photographs of plants taken before and after CO_2 measurements. Photosynthetic and respiratory activity of plants was expressed in μg CO_2 m^{-2} s^{-1}. The respiratory activity of earthworms was expressed with respect to the mass of organisms measured before CO_2 measurements in μg CO_2 $g^{-1}h^{-1}$.

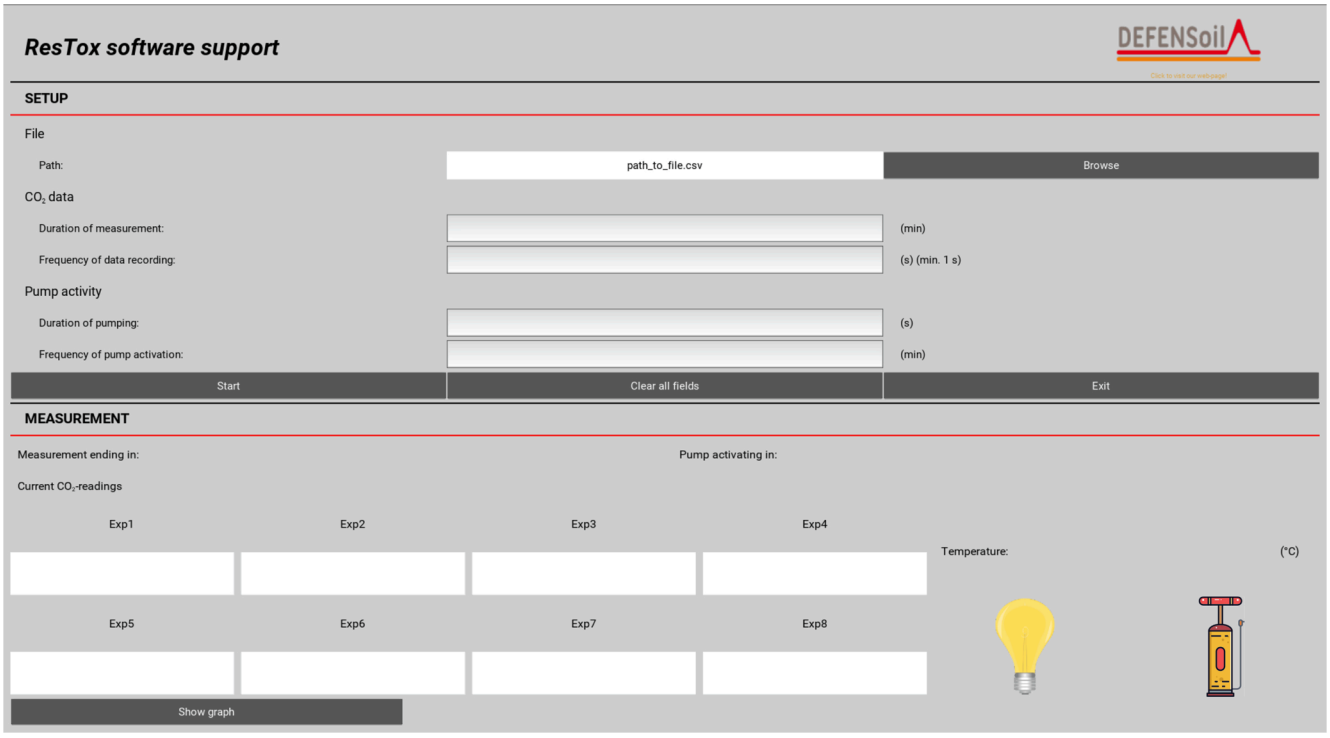

Fig. 2. Screenshot of the software support accompanying the developed sensor system.

2.3.1. Plant organisms

The CO_2 production of duckweed (*Lemna minor* L.) was measured to test the ResTox system's performance in experiments involving plant material. A detailed description of the experimental procedure is found in Peršić et al. [15]. Briefly, plants were placed in Erlenmeyer flasks (volume 100 mL), in 50 mL of standard growth medium [27] in which various concentrations of the chosen toxicants (four metals (Cu, Co, Cd, and Hg) and four herbicides (tembotrione, diquat, clopyralid, and nicosulfuron)) had been added. Test vessels were kept in conditions of light and dark cycles (3 h light – 3 h dark) and incubated for 24 h.

2.3.2. Animal organisms

The ResTox system's performance in experiments with animal organisms was tested by measuring CO_2 production in earthworms (*Dendrobaena veneta*) within filter paper and artificial soil experimental setups.

A filter paper test was conducted with a modified OECD Guideline [28]. Namely, the moistened filter paper was placed at the bottom of the Erlenmeyer flasks (250 mL). For a reference sensor and the control group, only distilled water was applied on the filter paper, while the treatments included three sublethal concentrations of the insecticide chlorpyrifos – 0.0795 μg cm^{-2} (C1), 0.795 μg cm^{-2} (C2), and 2.384 μg cm^{-2} (C3). In each test chamber, five earthworms (total weight of approximately 5 g) were added. The reference chamber with the reference sensor contained no earthworms. All test chambers were kept for 12 h in the dark at 20 °C. Experiments were run in three replicates.

For the artificial soil experiment, the CO_2 production of earthworms exposed to an organophosphate pesticide was measured. Measurements were conducted in closed glass chambers (500 mL) filled with 400 g of artificial soil prepared according to the OECD Guideline [28], with soil moisture adjusted to 50% water holding capacity. Earthworms were exposed to three concentrations of chlorpyrifos – 0.5125 mg kg^{-1} (C1), 2.56 mg kg^{-1} (C2) and 7.68 mg kg^{-1} (C3). In the control group and the chamber containing the reference sensor, only distilled water was added, and no earthworms were added in the reference test chamber. Test chambers were kept for 24 h in the dark at 20 °C. Experiments were run in three replicates. A 12 V vacuum pump was built into the developed system to facilitate the controlled removal of air from the test chambers. The pump activity effect was tested within an artificial soil setup by placing a tube connected to the vacuum pump inside each test chamber. The ResTox instrument was controlled through the accompanying software (Fig. 2) and programmed to activate the pump every 120 min with a duration of 120 s.

2.3.3. Data analysis

Statistical analysis of data was performed in R version 4.0.2 [29]. Results of experiments involving duckweed were analyzed, as described in Peršić et al. [15]. Briefly, measurements of CO_2 uptake/emission were fitted to a logistic model, and data were transformed by linearization based on the logistic model's slope coefficient. Slopes of regression lines among various treatments were analyzed by employing the analysis of covariance (ANCOVA) followed by Tukey's HSD test. Data regarding the respiratory activity of earthworms in filter paper experimental setup were first tested for normality using the Shapiro-Wilk test, and homogeneity of variance was confirmed with Levene's test. As no significant differences from normality were detected, data were analyzed using one-way ANOVA followed by Tukey's HSD test. The presented sensor system comprises a vacuum pump that automatically removes air saturated with CO_2 from the test chambers; therefore, the earthworms' CO_2 emission curves include multiple consecutive CO_2 generation intervals. Respiratory activity curves were first segmented into 12 two-hour CO_2-generation segments (Supplementary Material S4). Respiratory activity was then calculated for each segment. As heteroscedasticity was detected, data were analyzed using the Kruskal-Wallis rank test, followed by multiple pairwise-comparison between groups using the Wilcoxon rank-sum test.

3. Results and discussion

Sensor readings collected by the developed sensor system were successfully logged on a computer. Measurement parameters, such as measuring time, frequency of data logging, and pump activity, were also

set up using the software, which has proven to be a simple communication interface between the user and the ResTox device. In addition to hardware control, the software also allows real-time visualization of sensor readings during measurements. Data on CO_2 concentration in each experimental chamber and temperature and light intensity were successfully stored in .csv and .rtox log file-pairs, in standardized formats. Due to their structure, these files can easily be loaded into various data analysis and spreadsheet programs.

3.1. The sensor outputs correction and validation

Log data in .rtox files (Supplementary Material S5) indicate that the CO_2 sensor correction algorithm successfully levels outputs of experimental sensors based on reference sensor readings. Experiments were not performed in a room with constant and controlled levels of CO_2; furthermore, a continuous drop of CO_2 was reflected in the readings of the reference and experimental sensors. This obstacle was overcome by implementing the correction algorithm presented here (Equations 1–4). By installing a reference sensor, noise resulting from changes in the experimental setup's surroundings was eliminated, and sensor readings, preprocessed according to Equations 1–4, reflected only the effects of the test substance on CO_2 production/uptake of the examined organisms. Consequently, this system shows great potential for extending its application in measurements conducted in non-standardized conditions, such as those observed in the field.

During the validation test, sensor readings followed a linear trend regarding increasing reference CO_2 concentrations ($R^2 = 0.995$) (Fig. 3). Total sensor readings' error, expressed as RMSE, was 41.8315 mg kg^{-1}, which is within the accuracy range defined by the manufacturer. A similar result is reported in Joshi Gyawali et al. [30], where a different model of the MH-Z16 sensor series (range 400–10000 mg kg^{-1}) was used as a low-cost alternative for soil respiration measurements. The mentioned research also demonstrated that their Arduino-based sensor system had similar performance as a commonly used commercial field-portable IRGA system (LI-COR 8100). Moreover, Pumpanen et al. [31], by comparing 20 chambers for measurement of soil CO_2 efflux against known CO_2 fluxes, showed that different chamber techniques allow estimation of CO_2 fluxes within an under- and over-estimation range of −21 to + 33% (depending on the chamber type and experimental setup).

Experiments involving the commonly used, commercial Solvita soil respiration test system yielded a linear relationship between daily CO_2-C emissions from several soil types determined using the Solvita system and the ResTox system (Fig. 4). However, our results are in accordance previous studies [32]. Additionally, an exponential relationship was observed between the CO_2 flux measured using the ResTox system and the Solvita numbers measured for soil samples (Supplementary Material S6), which is in accordance with the Solvita publications, technical documentation and manuals [33]. The proposed system, as opposed to other systems similar to Solvita, does not produce any waste since it comprises reusable and long-life CO_2 probes. It also allows review and analysis of continuous CO_2 uptake/emission dynamics and insight into the exact experimental conditions (i.e. CO_2 concentration in the experimental setup's surroundings at the beginning and during the tests). Moreover, the NDIR sensors incorporated into the presented system have a relatively wide effective range (up to 50000 mg kg^{-1} CO_2), which allows accurate measurements without saturation over a much wider range compared to devices and methods from the same price category as the ResTox system. Finally, the developed system represents a direct method of CO_2 measurements, whose results are based on straightforward multipoint calibration (Fig. 3). Additionally, since it is based on the open-source Arduino platform, the ResTox system could also be the outset for a wide range of applications and future development.

3.2. Experiments with Lemna minor L

Data from experiments involving duckweed (Fig. 5) reflect light- and dark-phase changes, i.e., photosynthetic and respiratory activity of metabolically active plant tissues. CO_2 production and -consumption phase changes that can be distinguished by examining CO_2 concentration data are consistent with the light sensor's outputs. Closer examination of data also shows temperature changes coherent with light–dark phase exchanges. Detailed overview and discussion on results obtained in experiments involving duckweed can be found in Peršić et al. [15]. As an example, results obtained from experiments where plants had been exposed to various concentrations of Cu show a hormetic response of *Lemna* plants to the toxicant's presence, reflected in net photosynthesis and respiratory activity of plant tissue (Table 1).

The obtained results suggest an enhanced performance of physiological activities (respiration or photosynthesis rate) at the lowest concentrations of the applied toxicants. These results can be explained by an increase in energy uptake from the environment as a mechanism to counteract the effects of the toxicants and damage repair, which will

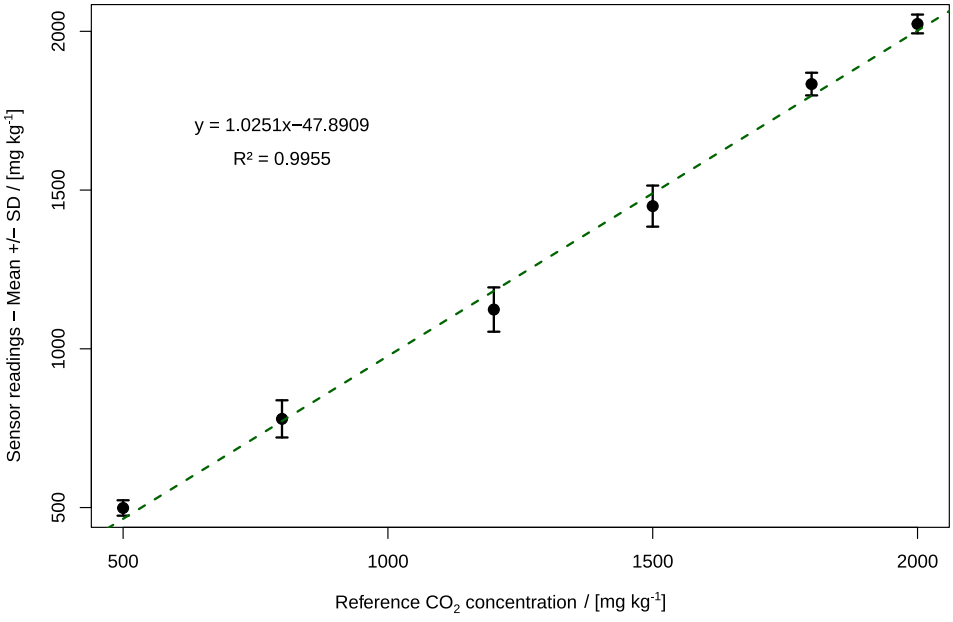

Fig. 3. Sensor readings (mean ± standard deviation of readings obtained from 9 NDIR CO_2 sensors) plotted against reference CO_2 concentrations in the chamber.

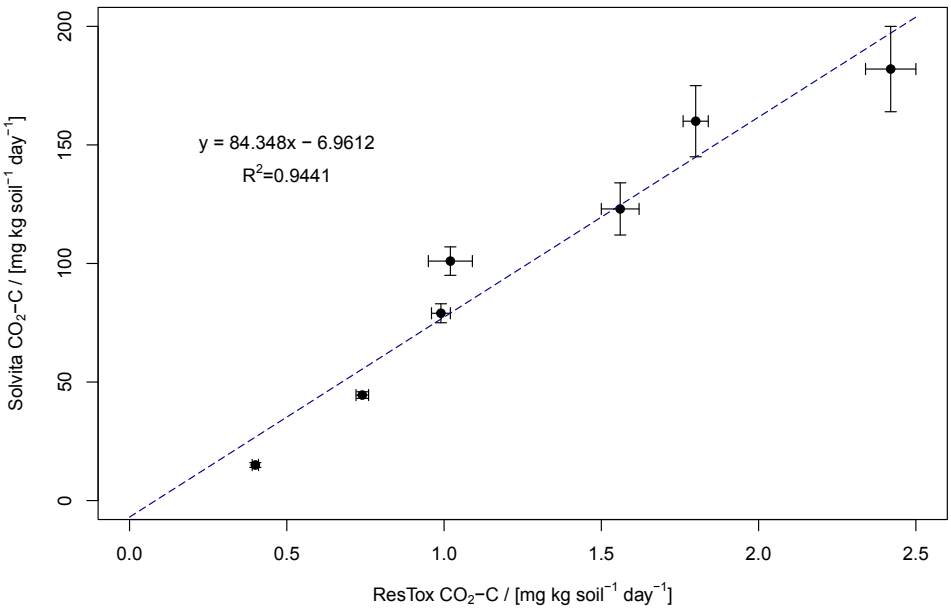

Fig. 4. The relationship between CO_2-C as determined using the Solvita soil respiration test system and the ResTox system.

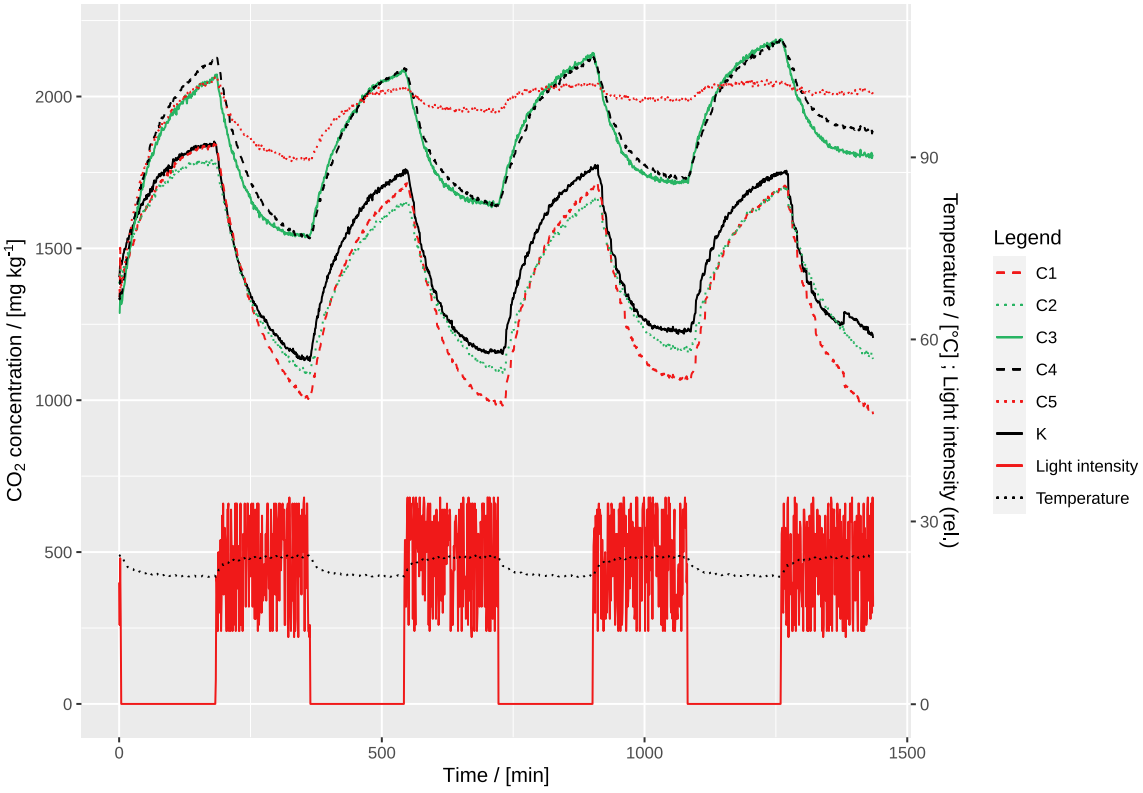

Fig. 5. Respiratory and photosynthetic activity of *Lemna* plants exposed to various concentrations of Cu, K denoting the control group and C1 to C5 growing concentrations of the toxicant.

subsequently increase the growth rate [34].

Although rarely studied, CO_2 exchange flux rate, representing the ratio between the photosynthetic and respiratory activity of duckweed reflected in CO_2 or O_2 uptake/emission, proves to be a valuable endpoint in standard phytotoxicity tests [14,15]. The lack of publications examining gas exchange in plants might be due to the complexity of the apparatus required for performing measurements (e.g., [35]), which, although showing high accuracy, are often expensive and thus inaccessible to most laboratories. Performing measurements presented here and in [15], the usability of the ResTox system, as an application of continuous monitoring of the emission and uptake rate of CO_2 in the Lemna toxicity test, was evaluated and confirmed. These results suggest the high accuracy of low-cost sensors incorporated into the system, making ResTox a promising tool in phytotoxicity test procedures.

Table 1
Average values with standard deviations of photosynthetic and respiratory activity of *L. minor* plants treated with various concentrations of Cu.

Cu concentration / [μg Cu mL^{-1}]	Net photosynthetic activity (mean ± SD) / [μg CO_2 m^{-2} s^{-1}]	Respiration rate (mean ± SD) / [μg CO_2 m^{-2} s^{-1}]
0	21.39 ± 3.07	15.67 ± 1.52
0.027	25.78 ± 3.88	22.42 ± 1.59
0.268	16.51 ± 1.65	14.68 ± 0.67
1.341	11.43 ± 1.81	9.98 ± 0.87
2.683	10.06 ± 2.67	9.75 ± 1.15
26.828	$6.44 \times 10^{-14} \pm 6.2 \times 10^{-14}$	2.40 ± 1.4

3.3. Experiments with Dendrobaena veneta

CO_2 production data obtained in experiments with earthworms exposed to chlorpyrifos in a filter paper setup (Fig. 6a) show a dose–response type relationship between the toxicant concentration and the amount of CO_2 produced. Calculated means and standard deviations of respiratory activity of organisms involved in this experiment were 12.248 ± 1.27 (C0), 8.81 ± 0.38 (C1), 23.85 ± 2.76 (C2), and 39.42 ± 5.59 μg CO_2 $g^{-1}h^{-1}$ (C3), respectively. Respiratory activity was significantly higher in C2 and C3 treatments when compared to control ($p < 0.05$) (Fig. 6a).

As for data on respiratory activity of earthworms exposed to chlorpyrifos in artificial soil (Fig. 6b), which presents a more realistic simulation of environmental conditions, a significant difference from the control group was found only in C3 treatment. Means and standard deviations of respiratory activity of earthworms during the exposure were 14.36 ± 1.19 (C0), 14.96 ± 0.93 (C1), 13.39 ± 3.47 (C2), and 23.28 ± 3.47 (C3) μg CO_2 $g^{-1}h^{-1}$, respectively.

Due to their anatomical and physiological features and sensitivity proven in exposure to xenobiotics, earthworms are considered valuable organisms in the research of toxicity mechanisms of pollutants and understanding their modes of action on an ecosystem-level [36,37]. Since respiratory metabolism of lumbricids was recognized as a sensitive indicator of exposure to pollutants [38] , CO_2 production has been measured in several types of research focusing on the effects of sublethal concentrations of toxicants on the physiology of earthworms [39,9]. The results obtained in both filter paper and artificial soil experiments are in accordance with several other authors' results. Namely, a stimulatory effect of pesticide exposure on the metabolic activity of earthworms has been recorded [38,39,9]. However, in contrast to discrete-time (point) measurements presented in previous research on earthworm respiration [40,38,39,9], the main advantage of the developed ResTox system is allowing highly accurate continuous monitoring of CO_2 release in various experimental setups, at a significantly lower price than some of the solutions presented in recent investigations [41].

Pump activity was successfully tested within an artificial soil experimental setup. Log data obtained during the measurements (Supplementary Material S4) proves the vacuum pump's efficiency in eliminating air saturated with CO_2, reflected in a simultaneous decrease of the concentration of this gas inside all test chambers. This feature could be useful in experiments where high CO_2 production is expected or, in the presented case, a rapid saturation of air contained in the sampling chamber is observed. Moreover, as in the presented research, pump activation also enables a kind of "reset" of conditions inside the chambers, allowing the performance of multiple consecutive measurements without the need for manual aeration or transfer of organisms to new chambers. Possible use of this feature of the system would be a more precise examination ofCO_2-regeneration dynamics of the studied organisms in the domain of time - in user-defined intervals and with customized pump activity settings.

3.4. Future directions

In its current configuration and thanks to the custom-made accessories and adapters (Supplementary Material S2), the developed system is adapted to continuous CO_2 monitoring applications performed in up to eight Erlenmeyer flasks or artificial soil-containing jars in parallel. The challenges posed by non-standard conditions (Supplementary Material S5) were overcome by implementing a self-correction algorithm presented in Equations 1–4 and contained in the microcontroller code (Supplementary Material S3). However, additional modifications and upgrades could further improve the system's performance and applicability. With the current system setup, ResTox supports data logging on a computer running the accompanying software. By upgrading the current system with a data logging shield supporting SD cards of different memory capacity and installing a solar power system, the ResTox system could be used as a compact, independent and autonomous field measuring device. With the experiments conducted, we demonstrated

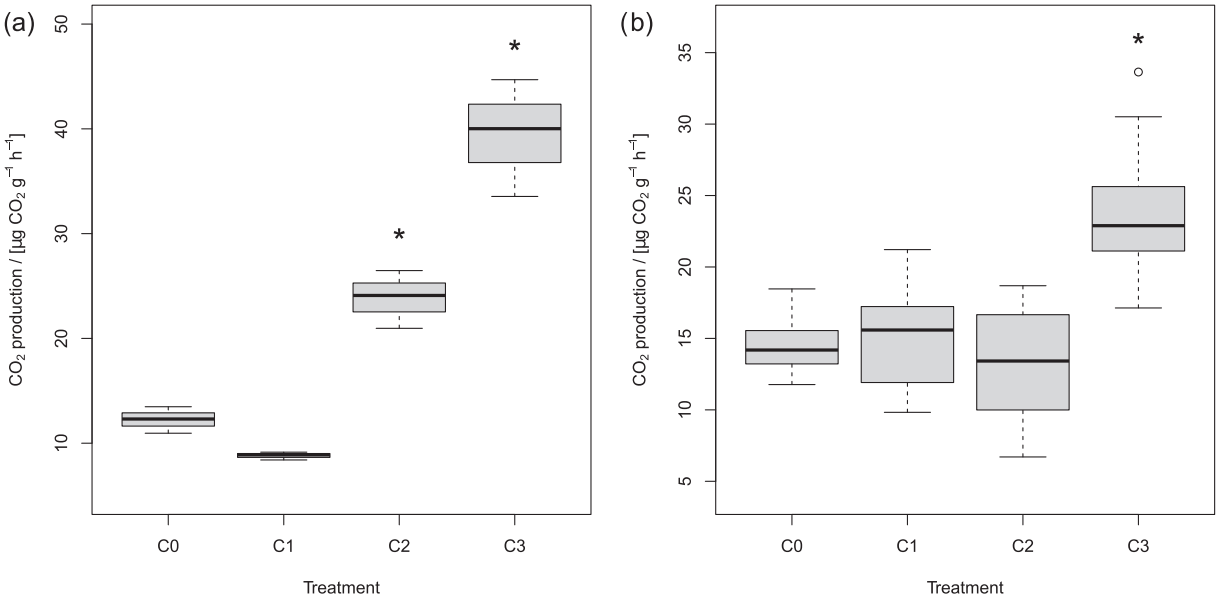

Fig. 6. Respiratory activity of earthworms in the control group (C0) and of those exposed to three concentrations of chlorpyrifos (C1-C3) in a filter paper (a) and artificial soil test (b). Statistically significant differences between treatment groups and control are represented by asterisks ($p < 0.05$).

that ResTox is suitable for measuring the metabolic activity of both plant and animal organisms, implying its applicability for other research, such as soil microbial activity. Similar low-cost solutions implementing NDIR CO_2 sensors described by Harmon et al. [17], Kaur et al. [18] and Joshi Gyawali et al. [30] pointed out the applicability of affordable sensors for soil quality determination through soil CO_2 efflux measurements. Based on Harmon et al. [17] , a possible extension of the current system setup could be a fan that would mix and homogenize the air in the experimental chambers and contribute to more precise gas concentration measurements.

Moreover, as the developed system is based on the popular open-source Arduino platform, it is extendable with virtually any sensor compatible with microcontrollers. By including other greenhouse gases (GHG) sensors (e.g., methane and nitrous oxide), the presented system could serve as a GHG monitoring station communicating continuous and real-time data at the request of the user. Thus, the proposed system, with minor modifications in the form of the addition of other GHG sensors, could potentially be used as a tool in climate change research and investigation of biogeochemical cycles [42–44].

4. Conclusions

ResTox is a simple, inexpensive, easily replicable respiratory activity measuring system allowing highly accurate continuous and real-time measurement of CO_2 dynamics in various experimental setups. Due to its customizability, this system may interest a diverse audience of users that could contribute to its improvement and specialization in numerous applications.

CRediT authorship contribution statement

Tamara Djerdj: Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization. **Vesna Peršić:** Methodology, Validation, Formal analysis, Investigation, Writing – original draft. **Davorka K. Hackenbergera:** Conceptualization, Writing – original draft, Project administration. **Domagoj K. Hackenberger:** Methodology, Software, Data curation, Writing – original draft. **Branimir K. Hackenberger:** Conceptualization, Methodology, Validation, Formal analysis, Resources, Writing – original draft, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Authors' contributions

BKH and DaKH conceived the ideas; TD, VP, and DoKH designed and realized methodology; TD, DaKH, and BKH led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Appendix A. Supplementary material

Source code of the ResTox application: https://github.com/gyorgytamara/restox-software. Supplementary data to this article can be found online at https://doi.org/10.1016/j.measurement.2021.109928.

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