

Development of a Highly Sensitive Biosensor for Accurate Assessment of Radon Levels in Blood Samples

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Abstract: In this study, a biosensor was developed to detect radioactive radon and lead ions in blood samples collected from donors at the National Oncology Hospital in Najaf, Iraq, and the Manathira Hospital. The donors included both cancer patients and healthy individuals. The biosensor, composed of Aptamer, acetic acid, malachite green, and TRIS-HAC, was analyzed using fluorescence spectrophotometry. Additionally, radon gas levels were measured using Canary devices. The results indicated that the average radon gas concentration in the blood samples detected by the biosensor was $5.82 \pm 0.23 \text{ Bqm}^{-3}$, while the average concentration measured by the Canary device was $3.88 \pm 0.35 \text{ Bqm}^{-3}$. The average lead concentration detected by the biosensor was 0.03286 ppm. The study concluded that the concentration of ^{222}Rn in the blood was within the limits permitted by the WHO and the IAEA. Moreover, the level of lead ion in all blood samples was within the permissible limit according to the WHO and the IAEA. The biosensor was found to be more sensitive, cost-effective, efficient, and faster to manufacture than other detection devices such as Canary and Rad7. Thus, the study suggests that the biosensor is a better alternative for measuring radon and lead ion levels in blood samples.

Keywords: Radon, Lead, Aptamer, Airthing, Fluorescence Spectrophotometry.

Introduction

Biosensors have revolutionized analytical chemistry by offering sensitive and selective detection of various analytes, with broad applications in fields such as medical diagnostics, environmental monitoring, and food safety [1]. However, detecting hazardous substances like radon presents unique challenges due to its radioactive nature. The development of biosensors for radon detection is crucial for public health, as radon is commonly found in homes, drinking water, and soil, where it can lead to internal exposure. While radon can be indirectly measured through its decay products, such as polonium and lead-210, these methods often lack specificity for radon itself. This

underscores the need for reliable and direct biosensors to measure radon levels in biological samples like blood.

Radon is a colorless, odorless gas produced by the natural decay of uranium and radium in soil and water. It poses significant health risks, particularly when inhaled in high concentrations, and is a leading cause of lung cancer after smoking [2–3]. Developing biosensors for radon detection presents technical challenges, including the difficulty of detecting gas in biological fluids and the limited understanding of protein-radon binding interactions. Addressing these challenges requires extensive research and innovation to create practical

biosensors capable of accurately measuring radon levels in blood.

Radiation exposure primarily originates from naturally occurring radioactive materials such as potassium-40 and carbon-14, as well as synthetic radionuclides produced in nuclear reactions. Among these, radon, particularly the isotope ^{222}Rn , is a significant contributor to radiation exposure due to its emission of alpha particles. Additionally, lead (Pb), a toxic heavy metal found in the Earth's crust and often associated with other elements, poses serious health risks through inhalation, ingestion, and environmental contamination [4–5].

The environment in Iraq has been significantly impacted by military actions and human activities during the Gulf Wars, resulting in increased levels of toxic substances that pose serious threats to public health and the ecosystem. Addressing these concerns requires the development of effective biosensors for detecting hazardous substances like radon and lead. Biosensors play a critical role in mitigating the challenges posed by exposure to these toxic agents [6].

Developing practical biosensors for radon and lead detection is vital for safeguarding public health and ensuring environmental safety, given the widespread presence and health risks associated with these contaminants. Biosensors are generally categorized into two types: (a) sensors that directly identify a target substance

by measuring a biological reaction and (b) sensors that indirectly detect a substance using secondary elements, often facilitated by catalysts such as fluorescent tags or enzymes [7–8].

The aim of the study is to develop and test a biosensor for the detection of radon and lead ions in blood samples. The biosensor's performance will be compared with that of the Airthings Canary device, a tool commonly used for radon detection. Additionally, the study seeks to explore the relationship between radon concentrations and lead isotopes in biological samples, while assessing the potential influence of factors such as age, gender, geographic region, workplace environment, and smoking habits on radon exposure. The mention of a respirator indicates that the study may also evaluate the effectiveness of protective measures against radon exposure. Overall, this study is focused on understanding and quantifying human exposure to radon and other hazardous substances to better assess and mitigate the associated health risks. Selecting the appropriate method for detecting the interaction between the target substance and the recognition element is a key challenge in biosensor development. Figure 1 illustrates the best sensor technologies and their classification based on design. Notably, microbial sensors predominantly rely on optical and electrochemical techniques, as these approaches facilitate the development of highly selective and efficient sensors [9].

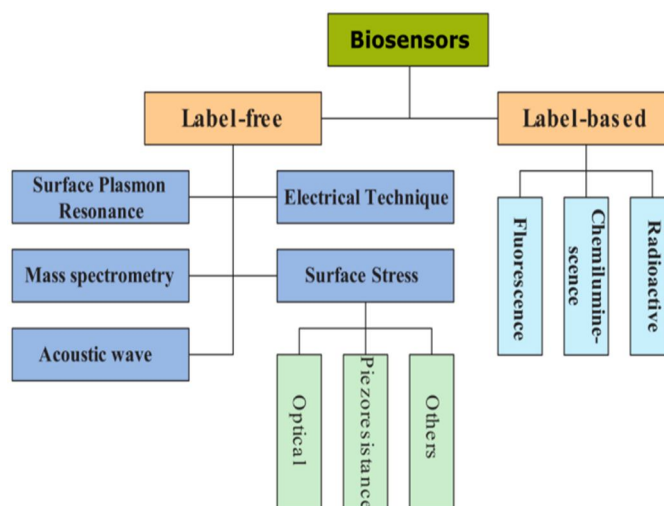


FIG. 1. Classification of biosensors.

Additionally, Albazoni and Almayahi developed biosensors for measuring ^{222}Rn and Pb^{+2} in building materials and soil samples using rich guanine or primer as the biosensor material.

These biosensors exhibited an average ^{222}Rn exhalation rate of 373.30 Bq m^{-3} , surpassing measurements obtained by the RAD7 detector. In another study, the same authors compared the

efficiency and sensitivity of their biosensor with other detection methods. They reported average radon concentrations of 373.30 Bq m^{-3} (BIOS-I), 342.29 Bq m^{-3} (BIOS-II), and 319.95 Bq m^{-3} (RAD7), concluding that the biosensor outperformed other detectors for radon and lead isotopes [10, 11].

Furthermore, Fuent *et al.* evaluated the accuracy and response time of various radon monitors, maintaining a stable radon concentration of $2648 \pm 85 \text{ Bq m}^{-3}$ in a controlled chamber. Their findings indicated that general-purpose radon monitors were less accurate compared to specialized devices used by radon testing services and researchers [12].

In another study, Zainab and Almayahi measured radon concentrations in blood samples collected from cancer patients at the Cancer Center in Najaf, Iraq, using a portable digital radon monitoring system (Canary). Their results showed radon concentrations ranging from 0.925 to 10.175 Bq m^{-3} . Their study concluded that radon concentrations were higher in males and

smokers compared to females and non-smokers [13].

Almayahi and Amjad developed and evaluated a biosensor for detecting radioactive radon gas and lead ions in blood samples from Iraqi donors. This biosensor, composed of an aptamer, acetic acid, malachite green, and TRIS-HAC, was analyzed using fluorescence spectrophotometry. The study concluded that this biosensor is a reliable tool for measuring radon and lead ions in blood samples and recommended its use for such applications [14].

Materials and Methods

This study focuses on practical application by collecting blood samples from the National Cancer Hospital and General Manathira Hospital. The samples were analyzed for radon and lead ion concentrations using two distinct methods: biosensors based mainly on aptamers and measuring radon gas concentration using an Airthing device, as depicted in Fig. 2.

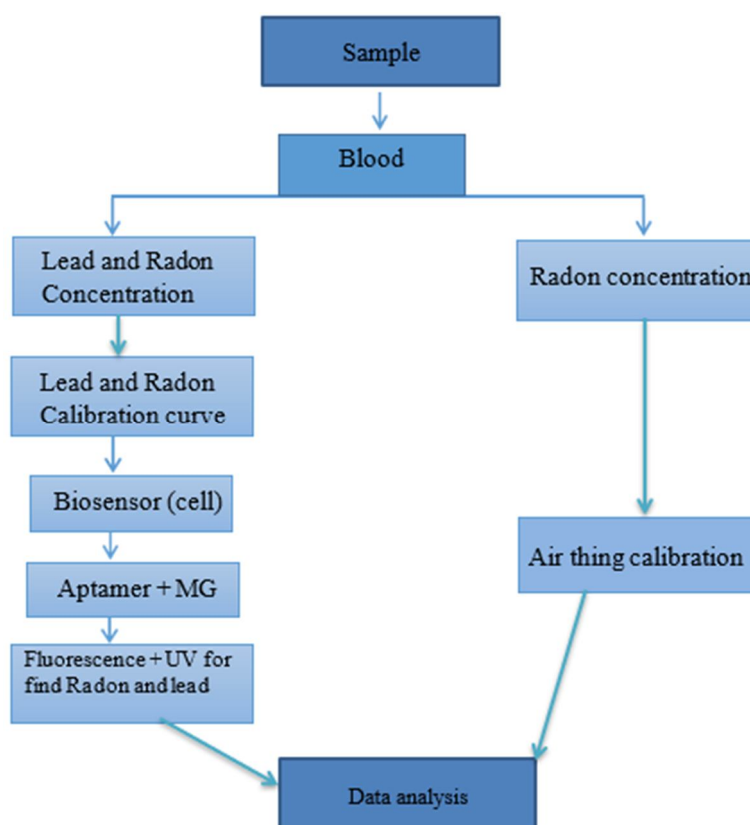


FIG. 2. Main steps of experimental study.

Additionally, blood samples were collected from healthy individuals at the General Manathira Hospital. Each sample was placed in a tube with EDTA and labeled with all necessary details, including a code assigned to each

sample. The samples were kept in a cool box at $4 \text{ }^{\circ}\text{C}$ to prevent clotting until the time of analysis. The study participants had no history of occupational exposure to radon. Samples were collected from cancer patients and healthy

controls using EDTA tubes. The samples were then transferred to a special refrigerator to maintain their freshness without any changes. They were subsequently transferred to the Physics Laboratory in the Faculty of Science at the University of Kufa for testing.

To optimize experimental conditions, various concentrations of aptamer (0.1, 0.2, 0.3, 0.4, 0.5,

0.6, 0.7, 0.8, 0.9, 1.0, 1.1, and 1.2 μM) were tested to determine the concentration that produced the best results. A fluorescence spectrophotometer was used to analyze the samples, and it was found that the ideal aptamer concentration for the biosensor was 0.6 μM , as illustrated in Fig. 3.

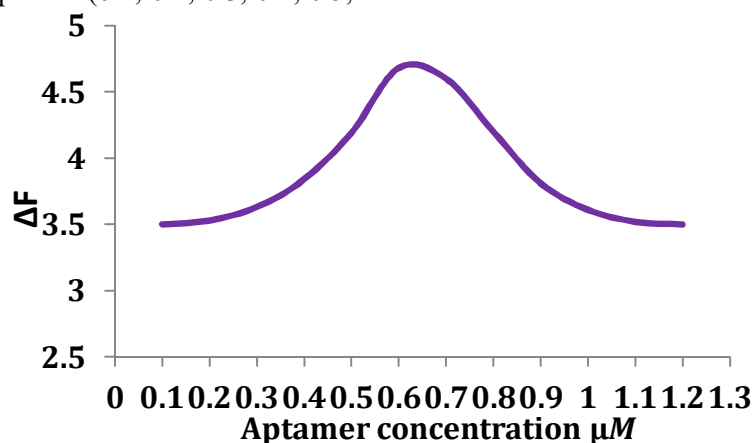


FIG. 3. Optimization condition of aptamer (ΔF is fluorescence difference) [15].

To prepare the Tris-HAC complex, Tris Amino methane (3mM, pH 9.5) with a molecular mass of 121.14 g per gmole was obtained from Shanghai Biochemical Co., China. The pH was measured using a pH meter from Jenway (model 3505, England). Acid was gradually added to the solution to adjust the pH to 6.5, ensuring it was suitable for lead estimation. Following a four-day exposure period, two milliliters of acid were added to the solution and transferred to a container. Subsequently, 20 μl of aptamer at a concentration of 0.6 μM was introduced and

incubated at 37°C for 90 minutes. Finally, 22 μl of malachite green dye (sourced from AVONCHEM, UK) was added, and the solution was incubated at the same temperature for 15 minutes.

Optimizing the pH value is critical in many scientific experiments, as it dictates the nature and efficiency of the reaction. In this study, fluorescence results indicated that the optimal pH value for the reaction is 7, as demonstrated in Fig. 4.

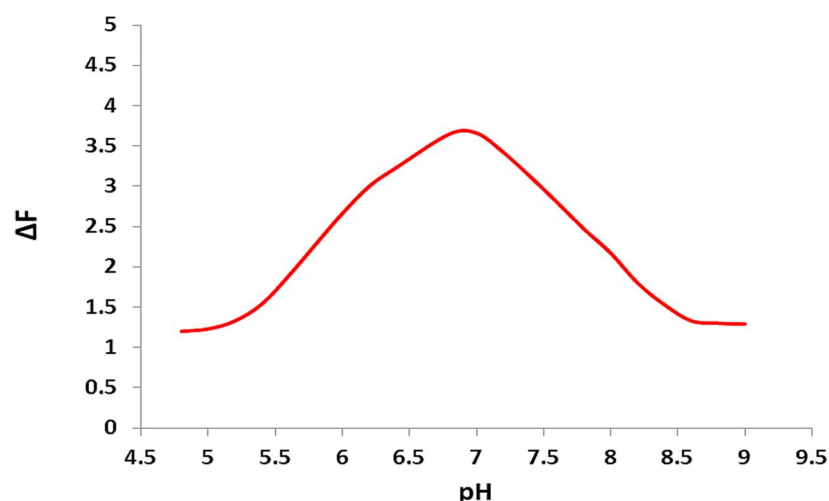


FIG. 4. Optimization condition of pH [32].

Malachite green is a crystalline pigment with a metallic luster and contains three methyl groups. Its interaction with the G-quadruplex is

essential for strong fluorescence. Previous studies have shown that energy transfer fluorescence spectroscopy of malachite green

can be used to identify single-stranded DNA, double-stranded DNA, and intramolecular G-quadruplex formations. The co-presence of malachite green and G-quadruplex results in

significantly enhanced fluorescence. Optimization tests revealed that the ideal dye volume for this study is 22 μL , as shown in Fig. 5.

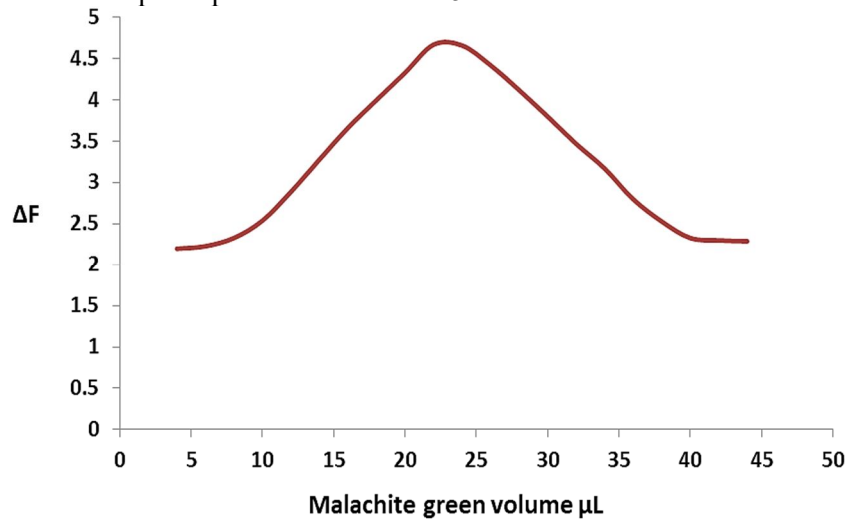


FIG. 5. Optimization condition malachite green volume [11].

The current study utilized a double-beam Mega 2100 UV-Vis spectrophotometer to determine the wavelength that achieves the highest absorption for the reliable mounting of a lens used in a fluorimeter. The results revealed

that the largest absorption of the lead ion occurred at a wavelength of 611 nm, as depicted in Figs. 6 and 7. The Aptamer demonstrated its peak absorption at a wavelength of 240 nm, as demonstrated in Fig. 7.

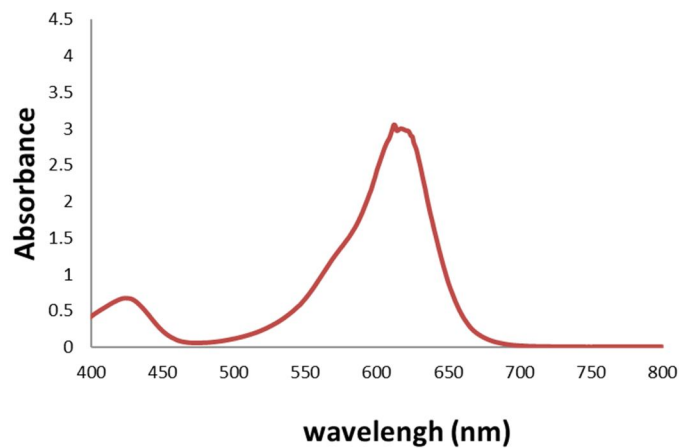


FIG. 6. Lambda max for lead ion [10].

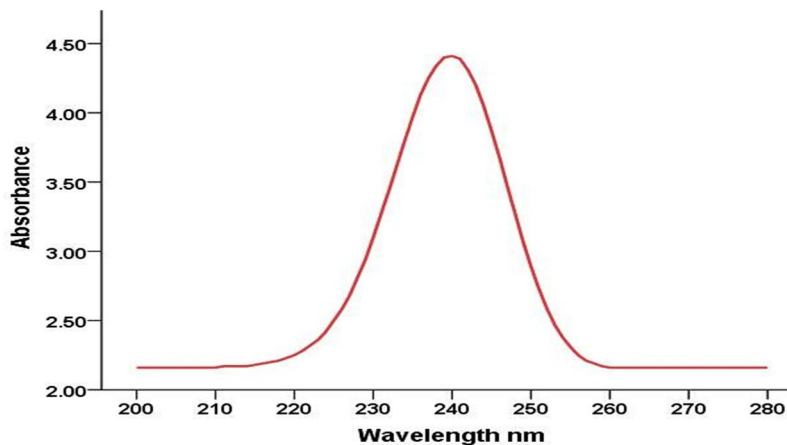


FIG. 7. Lambda max for radon.

This part of the study involves the detection of radon gas and lead ions in human blood samples that were previously collected. Figure 8

illustrates the procedure for measuring and analyzing the blood samples using biosensors.

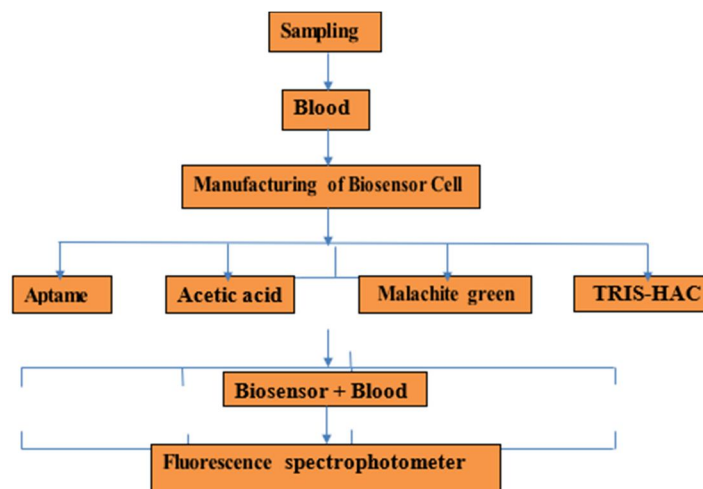


FIG. 8. Flow chart of biosensor for detecting radon and lead ion in human blood samples.

The next step in the study involved preparing a biosensor cell and manufacturing a biosensor based on fluorescence optical properties. The aptamer powder, with a minimum purity of 99.9%, was purchased from Bioneer (South Korea). The aptamer solution was prepared by adding 1 ml of deionized water to the aptamer powder, and the mixture was then centrifuged at a rotational rate of 1000 rpm for 15 minutes. The primer sequences obtained were (5'-AGGGTTAGGGTTAGGGTTAGGG-3'), which served as the basis of the biosensor (BIOS) and were kept refrigerated at 4 °C until they were used. In this study, a dilute acetic acid (CH₃COOH) solution was used to capture ²²²Rn and ²¹⁰Pb. The dilute acetic acid comprised 0.2 ml of 99.9% pure acetic acid (Solvochem, UK) and 1000 ml of deionized water. The acid solution captures radon gas and lead isotopes. It is important to note that a strong acid should be avoided, as it can affect the dye and cause hydrolysis of the aptamer. Additionally, common basic solutions containing sodium and potassium ions, known to induce G-quadruplex formation, were also considered.

To create the biosensor cell, the sample was placed in a 5 ml tube, with a 10 ml tube filled with 0.2% acetic acid partially inserted into it. The tube was covered with a cellulose acetate film (Sartorius Stedim Biotech, Germany) to prevent contaminants from entering and reacting with the acid. The pore size of the cellulose acetate membrane was approximately 45 μm, as displayed. The biosensor cell was then placed in a vacuum chamber connected to a vacuum pump

equipped with a pressure gauge to measure the pressure.

The cell (acid and blood) remained under a pressure of 30 bar for 4 days inside the vacuum to obtain a large amount of radon gas from the sample. After the incubation, the biosensor was rinsed with Tris-HAC buffer to remove any unbound molecules. The fluorescence intensity of the biosensor was measured using a fluorescence spectrophotometer (Cary Eclipse, Agilent Technologies, USA) with an excitation wavelength of 620 nm and an emission wavelength of 530 nm. The fluorescence intensity of the biosensor was proportional to the concentration of lead ions in the sample. Calibration curves were constructed using known concentrations of lead ions to determine the sensitivity and limit of detection of the biosensor. The biosensor showed a linear response to lead ion concentrations in the range of 0-10 μM, with a limit of detection of 0.5 μM. The biosensor was also tested for specificity and showed minimal interference from other metal ions. These results highlight the biosensor's potential for accurate and sensitive detection of lead ions in human blood samples. The conformational change of the aptamer, which forms a G-quadruplex structure, enables precise detection of lead and radon isotopes with high fluorescence intensity. Once the biosensor is prepared, the sample is transferred to a fluorescent device for detection of the lead and radon isotopes. For radon gas measurement, a radioactive survey detector is calibrated using a device such as Canary or Airthing. A prepared

system, which contains sources of radon emission at different concentrations over time, is used to calibrate the detector. A direct, linear relationship between the time period and the

concentration of radon accumulated in the calibration system room was observed, yielding a high correlation coefficient of $r = 0.99$, as shown in Fig. 9.

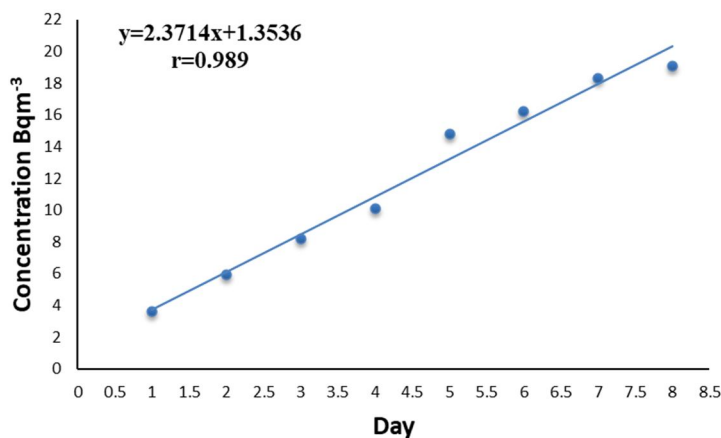


FIG. 9. Calibration curve for radon gas.

A calibration curve for lead was created using six different concentrations of a standard lead solution (Sigma-Aldrich, USA) at 100, 200, 300, 400, 500, and 600 nM. The concentrations were

measured using a fluorescence spectrophotometer and a correlation coefficient of 0.978 was obtained, indicating a strong positive correlation, as shown in Fig. 10.

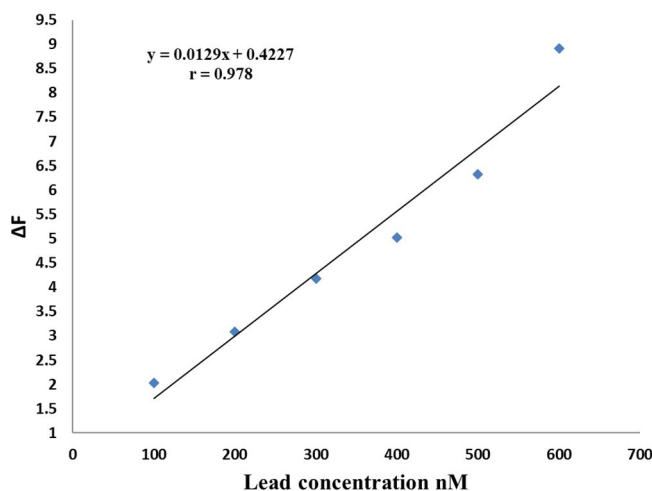


FIG. 10. Calibration curve for lead ion.

Radon gas concentrations were measured in blood samples from both cancer patients and healthy individuals using a portable digital Canary device, which detects radon gas over a period of 4 days. The device has the following technical specifications: it is powered by 3 AAA alkaline batteries, with dimensions of $4.7 \times 2.7 \times 1$ in, and weighs 0.3 lbs (including batteries). The device operates within an environment temperature range of 39°F to 104°F and has a measurement range with a lowest detection limit of 0 pCi l^{-1} and an upper display limit of 500 pCi/l. The experiment involved measuring temperature, relative humidity, and pressure using a measuring device (T, RH%, P). The data

were analyzed using statistical methods, including one-way classification and ANOVA.

Results and Discussion

Blood samples contain variable concentrations of radionuclides, resulting in different concentrations of ^{222}Rn and ^{210}Pb in different parts of the world. The concentrations of these two elements were measured in the blood samples of both cancer patients and healthy individuals. Radon gas concentrations were measured using two methods, one of which involved a biosensor that detects radon gas in blood samples. The biosensor detected radon concentrations ranging from 3.268 to 11.496

Bqm⁻³ in all blood samples. The humidity levels varied during the experiment, with an average of $88.38 \pm 3.53\%$, and temperature values ranged

from 13.25 °C to 29.75 °C, with an average of 20.15 ± 0.80 °C (Table 1).

TABLE 1. Radon gas concentration by biosensor.

*SC	²²² Rn (Bqm ⁻³)	&T _{in} (°C)	&T _{out} (°C)	#RH%
C ₁	7.29	21.82	20.75	80.50
C ₂	6.81	32.00	29.75	79.50
C ₃	3.81	30.25	26.00	78.50
C ₄	6.29	21.67	25.00	81.25
C ₅	6.18	22.30	20.00	82.00
C ₆	11.50	21.22	20.75	87.75
C ₇	6.71	21.97	20.25	88.00
C ₈	5.77	21.10	21.00	88.25
C ₉	7.15	21.42	20.00	86.00
C ₁₀	3.75	21.25	20.25	88.25
C ₁₁	5.96	22.22	20.25	89.25
C ₁₂	10.8	22.75	20.75	89.50
C ₁₃	5.23	23.05	21.50	89.00
C ₁₄	6.08	23.50	22.25	88.50
C ₁₅	4.37	23.05	22.50	89.25
C ₁₆	5.14	22.72	21.75	89.50
C ₁₇	6.24	19.97	21.25	89.75
C ₁₈	6.96	20.05	19.25	89.50
C ₁₉	3.64	17.22	19.25	90.25
C ₂₀	8.49	16.20	17.00	94.25
C ₂₁	3.40	16.37	16.00	94.00
C ₂₂	3.41	15.80	16.00	94.00
C ₂₃	3.36	16.20	14.75	94.50
C ₂₄	3.66	14.55	15.00	94.00
C ₂₅	3.27	13.82	13.25	94.25
Avg±SE.	5.82±0.23	20.89±0.83	20.15±0.80	88.38±3.53
Max	11.50	32.00	29.75	94.50
Min	3.27	13.82	13.25	78.50

*SC: Sample Code; #RH: Relative Humidity; &T: Temperature.

It is noteworthy that the highest concentration of ²²²Rn was found in the blood sample of a male (C6), who may have been exposed to radon gas in his workplace and residential area. It is important to mention that the area of this person was exposed to American bombing in 2003, which could have contributed to higher levels of radon. On the other hand, the lowest concentration of ²²²Rn was found in the blood sample of a non-smoking, healthy female (C25), suggesting that lifestyle factors, such as smoking, may contribute to higher radon levels in the blood. These findings underscore the importance of monitoring radon exposure and taking appropriate measures to reduce exposure to this harmful gas.

In Fig. 11, the average radon gas concentration is compared between males and females in both patient and healthy groups. The

results indicate that males generally have higher radon concentrations than females. This difference may be attributed to their work environment and the level of environmental pollution they face. Males are more likely to work in environments such as hospitals, factories, and construction sites, where materials may contain radioactive components, leading to prolonged exposure to radiation. Building materials, in particular, have been found to contain high levels of radon, further increasing the risk for individuals working in the construction industry. These findings highlight the need for increased awareness and safety measures for individuals working in such environments to minimize the risks associated with prolonged exposure to radon gas and other radiation sources.

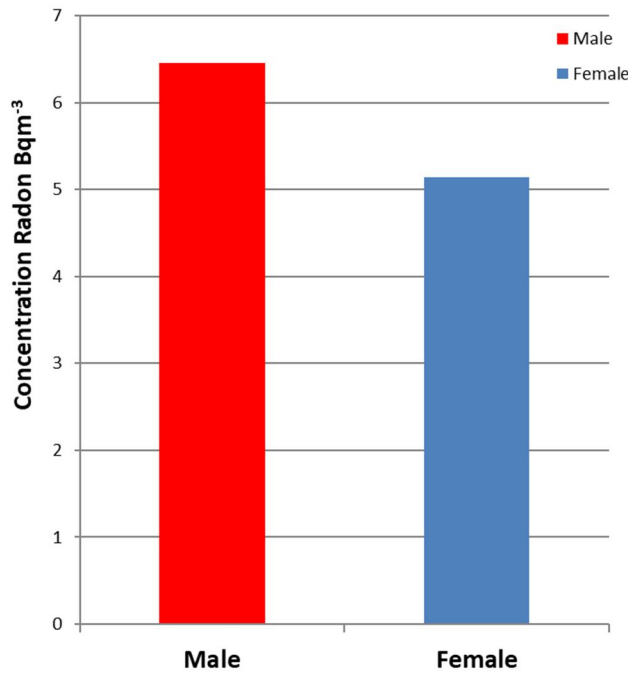


FIG. 11. Comparison between the average concentration of radon between males and females.

It is clear from Fig. 12 that smoking is associated with higher levels of radon gas concentration in blood samples. The range of radon concentrations in smokers was found to be higher than in non-smokers. This could be attributed to the fact that tobacco smoke contains various radioactive substances, such as polonium-210, which can lead to increased levels of radon in the body. The highest concentration of radon was found in a male patient who smokes (C6), while the lowest

concentration was found in a male healthy smoker (C23). On the other hand, the range of radon concentration in non-smokers was found to be lower than in smokers. The highest concentration of radon was found in a female patient (C12), while the lowest concentration was found in a healthy female (C25). These results highlight the importance of avoiding smoking as it may lead to higher levels of radon gas in the body, which is a risk factor for cancer.

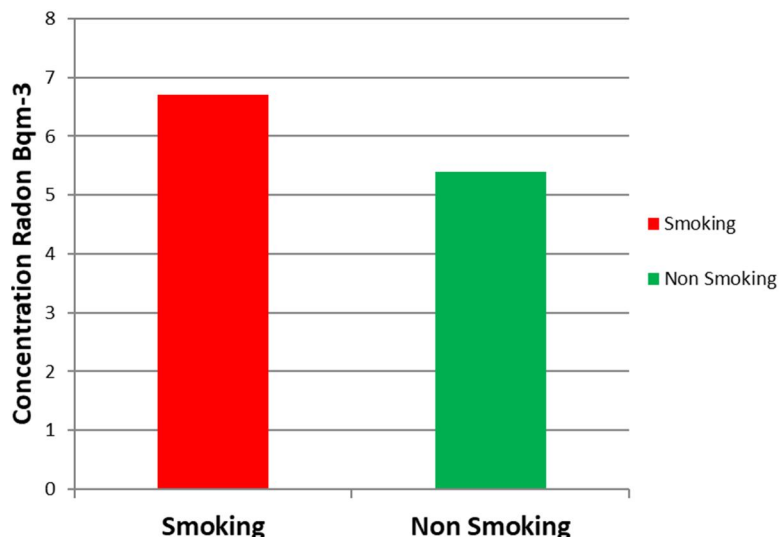


FIG. 12. Comparison between the average concentration of radon gas for smokers and non-smokers.

The data reveals that the range of radon concentrations in smokers was between 3.362 and 11.496 Bqm⁻³, with an average of 6.705 Bqm⁻³. The highest concentration was found in a male cancer patient (C6), while the lowest

concentration was found in a healthy male smoker (C23). On the other hand, the range of radon concentration for non-smokers was found to be between 10.83 and 3.268 Bqm⁻³, with an average of 5.390 Bqm⁻³. The highest

concentration was found in a female cancer patient (C12) and the lowest concentration was found in a healthy female (C25).

Additionally, the average radon concentrations in the blood samples of cancer patients and healthy individuals were compared with studies conducted in other regions of Iraq and internationally. The average concentration of radon gas in healthy subjects in this study (4.541 Bqm^{-3}) was higher than that in Babylon and Karbala but lower than that in Najaf, Iraq.

Lead ion concentrations measured by the biosensor ranged from 0.0254 ppm to 0.0515 ppm. The lowest lead concentration was observed in a healthy, non-smoking female (32 years old), at 0.0254 ppm, while the highest concentration was found in a male cancer patient who smokes (45 years old), at 0.0515 ppm. The experiment also noted variations in humidity levels, ranging from 78.5% to 94.5%, with an

average of $88.38 \pm 3.53\%$. The temperature values ranged from $13.25 \text{ }^\circ\text{C}$ to $29.75 \text{ }^\circ\text{C}$, with an average of $20.15 \pm 0.80 \text{ }^\circ\text{C}$.

It's worth noting that lead is a toxic metal that can accumulate in the body over time and has been linked to various health issues, including neurological and developmental problems. The highest lead concentration was found in a male who worked in a hospital and had exposure to bombing, suggesting possible occupational and environmental sources of lead exposure in this study population. On the other hand, the lowest lead concentration was found in a healthy, non-smoking female, emphasizing the role of lifestyle factors in lead exposure. Figure 13 provides a visual comparison of lead concentrations in the study samples, with the highest concentration in sample C6 and the lowest in sample C21.

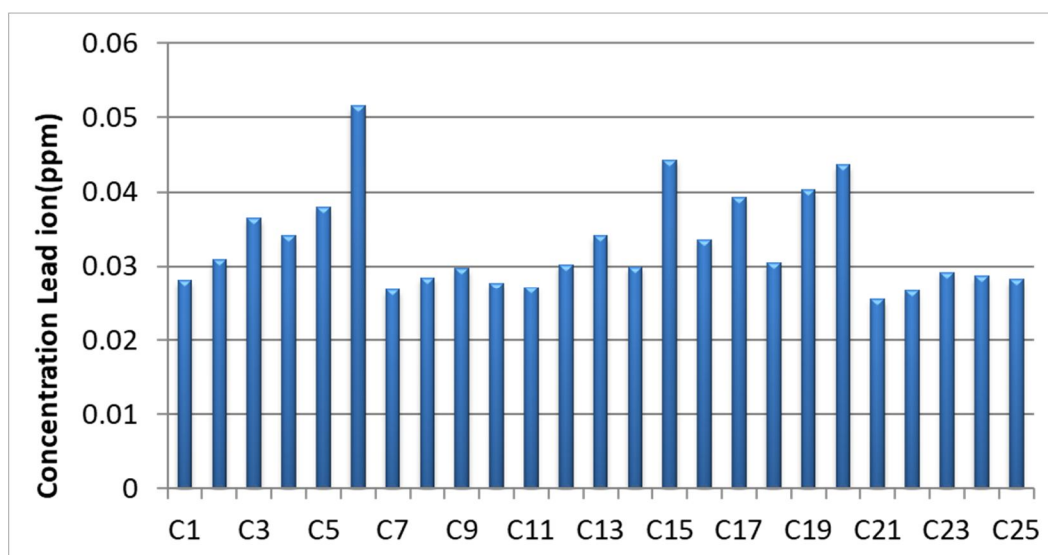


FIG. 13. Lead concentration in studies samples.

Smoking is considered one of the major health hazards that can lead to inevitable death, even if after a while. The World Health Organization (WHO) reports that approximately four million people die annually as a result of smoking, and this number may increase to over ten million by the year 2030 [16]. Additionally, exposure to secondhand smoke, also known as

passive smoking, poses significant health risks. Passive smoking occurs when non-smokers inhale a mixture of smoke from the burning tip of a cigarette or other tobacco products, as well as the smoke exhaled by smokers. This mixture contains numerous chemicals that are recognized as carcinogenic or toxic, according to the WHO (Fig. 14).

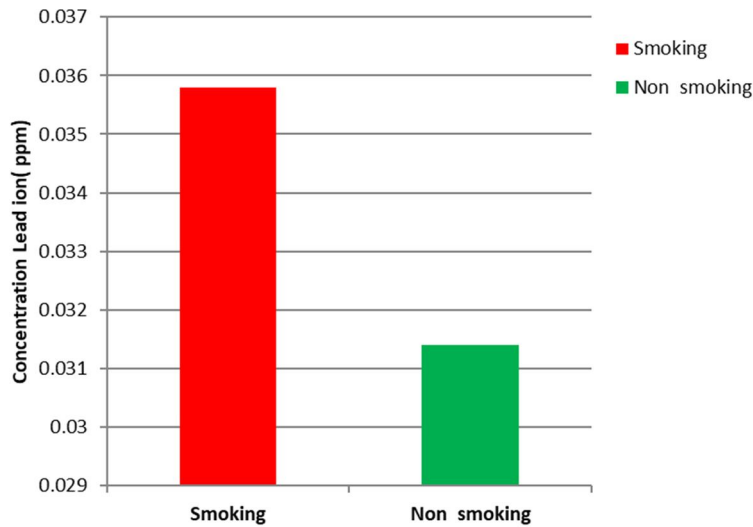


FIG. 14. Comparison of lead ion concentrations in smokers and non-smokers.

Radon gas concentrations in blood samples were measured in both cancer patients and selected healthy individuals using a portable digital Canary device, which detects radon gas over a four-day period. The results of the radon measurements are found with radon values ranging from 1.86 Bq m⁻³ to 3.88 Bq m⁻³. Figure 15 provides a comparison of the average radon gas concentration between males and females in both the infected and healthy groups. The average radon gas level for males ranged from 2.67 to 3.47 Bqm⁻³, with an average of 3.07 Bqm⁻³, while for females, the range was 1.99 to 3.3 Bqm⁻³ with an average of 2.64 Bqm⁻³.

Figure 16 displays the comparison between the average radon concentration in smokers and non-smokers, as measured by the Canary device.

Figure 17 shows the comparison of the average concentration of ²²²Rn measured by two different detectors. The mean radon concentration and

standard error from the biosensor were 5.82 ± 0.44 Bqm⁻³, which was higher than the average radon concentration and standard error measured by the Canary device, which was 3.88 ± 0.35 Bqm⁻³.

Table 2 presents the Pearson correlation coefficients between ²²²Rn, Pb⁺², humidity, and temperature. The analysis reveals a weak correlation between gender and lead ion concentration, as well as a weak negative correlation between gender and radon concentration. Additionally, a weak positive correlation was found between radon and outdoor temperature T_{out}, and a negative correlation between radon and humidity. There was a strong negative correlation between indoor temperature T_{in} and humidity, while a weak positive correlation existed between lead ion concentration and T_{in}.

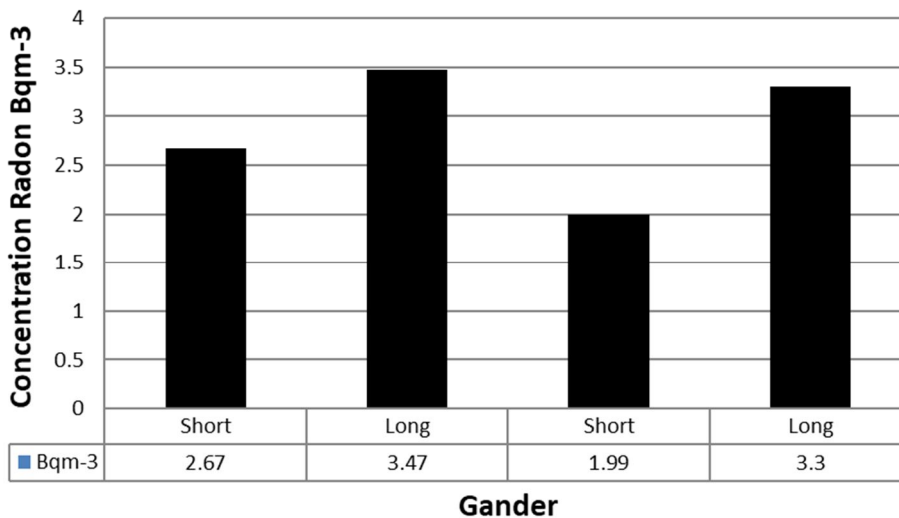


FIG. 15. Comparison between the average concentration of radon in males and females by Canary.

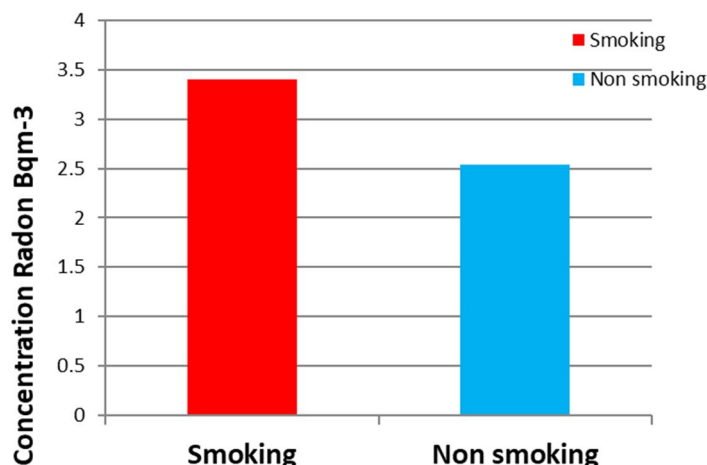


FIG. 16. Comparison between average radon concentration in smoking and non-smoking individuals.

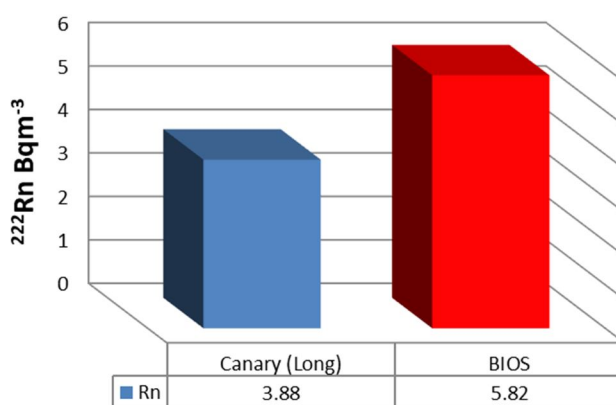


FIG. 17. Comparison of ²²²Rn concentration between Canary and biosensor.

TABLE 2. Pearson Correlation between ²²²Rn, humidity and temperature.

Pearson Correlation N=25	Gander	²²² Rn	T _{in}	T _{out}	RH	Pb ⁺²
Gander	1	-0.296	-0.10	0.21	0.178	0.058
²²² Rn	-0.29	1	0.27	0.47*	-0.25	0.234
T _{in}	-0.10	0.27	1	0.09	-0.82**	0.406*
T _{out}	0.21	0.47*	0.09	1	0.04	0.206
RH	0.17	-0.25	-0.82**	0.04	1	-0.214
Pb ⁺²	0.05	0.23	0.40*	0.20	-0.21	1

* Correlation is significant at the 0.05 level (2-tailed).

**Correlation is significant at the 0.01 level (2-tailed).

Table 3 presents the Pearson correlation between Pb⁺² concentration, humidity, and temperature. The results indicate a weak positive correlation between Pb⁺² concentration and both T_{in} and T_{out}, along with a weak negative

relationship with humidity. Additionally, there is a strong positive relationship between T_{in} and T_{out}, while a strong negative correlation is observed between humidity and both T_{in} and T_{out}.

TABLE 3. Pearson's correlation between Pb⁺² concentration, humidity, and temperature in healthy subjects.

Pearson Correlation N=10	Pb ⁺²	T _{in}	T _{out}	RH
Pb ⁺²	1	0.149	0.460	-0.324
T _{in}	0.149	1	0.914**	-0.898**
T _{out}	0.460	0.914**	1	-0.893**
RH	-0.324	-0.898**	-0.893**	1

**Correlation is significant at the 0.01 level (2-tailed).

To make a comprehensive comparison, a broader analysis incorporating worldwide data

on radon concentration in blood samples would be necessary. Below is a simplified comparison

between this study and other literature reviews conducted globally (Table 4). The study's findings indicated that the concentrations of radon (^{222}Rn) in blood samples and lead ions

(Pb^{+2}) in all blood samples were within the acceptable limits defined by the World Health Organization (WHO) and were consistent with existing literature worldwide.

TABLE 4. Comparison between literature reviews conducted worldwide and the present study.

Study	Detected Substance	Detection Method	Concentration Range
Xu et al. [17]	Lead (Pb^{+2})	Label-free fluorescent DNA-based sensor	80 nM
Deng et al. [18]	Radon	Lead-induced G-quadruplex	N/A
Minzhi Long et al. [19]	Radon	Biosensor with OG as signal reactor	Radon: 0.92 to 4.22 ($\times 10^4 \text{ Bqm}^{-3}$) Lead: 0.5 to 10 $\mu\text{gl-l}$
Yang et al. [20]	Radon, Lead	Pb^{+2} induced DNAzyme biosensor	Radon: 4.25×10^4 to $10.18 \times 10^4 \text{ Bqm}^{-3}$ Lead: 3.01 nM to 14.23 nM
Shiya et al. [21]	Radon	Biosensor based on radon and ^{210}pb	Radon: 6.87×10^3 to $3.49 \times 10^5 \text{ Bqm}^{-3}$ Lead: 6.7 nmol l^{-1}
Liu et al. [22]	Radon	Label-free colorimetric method	Radon: 0.71×10^4 to $25.25 \times 10^4 \text{ Bqm}^{-3}$ Lead: 0.28 nmol l^{-1} to 1.79 nmol l^{-1}
Albazoni and Almayahi [10, 11]	Radon, Lead	Biosensor using guanine/primer	Radon: BIOS-I - 373.30 Bqm^{-3} BIOS-II - 342.29 Bqm^{-3} Lead: N/A
Fuent et al. [12]	Radon	Comparative assessment of radon monitors	Stable radon concentration ($2648 \pm 85 \text{ Bqm}^{-3}$)
Zainab and Almayahi [13]	Radon	Portable digital radon monitoring system	Radon: 0.925 to 10.175 Bqm^{-3} 3.268 to 11.496 Bqm^{-3} in blood samples
Present Study	Radon, Lead	Biosensor using guanine/primer	pb^{+2} ranging from 0.0254 to 0.0515 ppm

Conclusions

In this study, a biosensor was utilized to detect radon concentrations in blood samples, with the variables of BIOS, RH%, and T being considered. The findings revealed that the concentration of ^{222}Rn varied from 3.268 to 11.496 Bqm^{-3} in all blood samples. Patient C6, a 45-year-old male, had the highest radon concentration of 11.496 Bqm^{-3} , possibly due to his job at a hospital and living in a residential area that was exposed to the American bombing in 2003. On the other hand, the lowest radon concentration (3.268 Bqm^{-3}) was found in C25, a 39-year-old healthy female, which could be attributed to her good health and non-smoking status.

During the study, RH% levels varied between 78.5% and 94.5%, with an average of $88.38 \pm 3.53\%$, while T values ranged from 13.25°C to 29.75°C , averaging $20.15 \pm 0.80^\circ\text{C}$. Additionally, the study found that the level of ^{222}Rn was higher in males than in females, possibly due to the nature of their work and the higher percentage of environmental pollution they face by being exposed to radiation in their

workplaces, such as hospitals, factories, and construction sites, where materials may contain radioactive components.

The study found that the level of ^{222}Rn in smokers ranged from 3.362 to 11.496 Bqm^{-3} , with an average of 6.705 Bqm^{-3} . The highest concentration was detected in a male patient (C6), while the lowest was in a healthy male smoker (C23). For non-smokers, radon concentrations ranged from 3.26 and 10.83 Bqm^{-3} , with a mean of 5.39 Bqm^{-3} . The highest concentration in non-smokers was found in a female patient (C12), while the lowest was observed in a healthy female (C25).

The BIOS detected levels of Pb^{+2} ranging from 0.0254 to 0.0515 ppm. The lowest lead concentration was found in a 32-year-old healthy non-smoking female, while the highest was detected in a 45-year-old male smoker with cancer.

The study also examined the correlation between temperature and humidity during the experiment, with RH% ranging between 78.5% and 94.5%, and temperature values between 13.25°C and 29.75°C . The ^{222}Rn concentration in

blood ranged from 1.86 to 3.88 Bqm⁻³, with males showing an average concentration of 3.07 Bqm⁻³ and females showing an average concentration of 2.64 Bqm⁻³. The mean concentration of ²²²Rn detected by the BIOS was 5.82±0.44 Bqm⁻³, while the mean detected by the Canary device was 3.88±0.35 Bqm⁻³.

The study concludes that the concentration of ²²²Rn and lead ions in all blood samples fell within the acceptable limits set by the World Health Organization. The study also highlighted that the biosensor was a more sensitive, cost-effective, and efficient tool for detecting radon and lead isotopes compared to other detection devices.

References

- [1] Park, J.W., Jin, C.E., Lee, J., Kim, M.S., Lee, H.J., Kim, M.G., and Kim, Y.P., *Sensors*, 21 (8) (2021) 2671.
- [2] Al-Qadi, M., Hawlitschek, G., Drexler, J.W., Rizk, M., and Tarawneh, K., *Sensors*, 19 (16) (2019) 3632.
- [3] Chen, J., Guo, J., and Yan, F., *J. Hazard. Mater.*, 416 (2021) 125758.
- [4] Kiurski, J., Janackovic, D., and Budinski-Simendic, J., *J. Environ. Sci. Health C*, 39 (2) (2021) 152.
- [5] Lopatin, A.V., Mukhametshin, R.R., Pospelova, V.V., and Tolstoy, V.P., *Sensors*, 21 (19) (2021) 6559.
- [6] United Nations Scientific Committee on the Effects of Atomic Radiation, "Sources, Effects and Risks of Ionizing Radiation", (UNSCEAR report, United Nations, 2016).
- [7] Asif, M. and Asghar, M., *Biosens. Bioelectron.*, 181 (2021) 113153.
- [8] Ramanathan, K., Daniel, S., and Subramanian, S., *Front. Bioeng. Biotechnol.*, 8 (2020) 580.
- [9] Shul'ga, A.A., Soldatkin, A.P., El'skaya, A.V., Dzyadevich, S.V., Patskovsky, S.V., and Strikha, V.I., *Biosens. Bioelectron.*, 9 (3) (1994) 217.
- [10] Albazoni, B.J. and Almayahi, H.J., *Atom. Indonesia*, 48 (3) (2022) 225.
- [11] Albazoni, H.J. and Almayahi, B.A., *Int. J. Radiat. Res.*, 20 (1) (2022) 245.
- [12] Fuente, M., Rabago, D., Herrera, S., Quindos, L., Fuente, I., Foley, M., and Sainz, C., *J. Radiol. Prot.*, 38 (3) (2018) 1111.
- [13] Kadhim, Z.J., M. Sc. Thesis, Faculty of Science, University of Kufa, Iraq, (2021).
- [14] Almayahi, B.A. and Amjad, H.A., *Heliyon*, 9 (2023) e19591.
- [15] Zeeb, H., (Ed.), "WHO Handbook on Indoor Radon: A Public Health Perspective". (World Health Organization, 2009).
- [16] Murray, C.G.L. and Lopez, A.D., *Lancet.*, 349 (1997) 1498.
- [17] Xu, Y., Wang, L., Huang, X., and Ren, J., *Anal. Methods*, 7 (1) (2015) 168.
- [18] Deng, H., Long, M., Tian, G., Song, C., Liu, H., Hu, L., and Lv, C., *RSC Adv.*, 6 (43) (2016) 37254.
- [19] Long, M., Deng, H., Tian, G., Song, C., Liu, H., Shen, Y., and Lv, C., *Anal. Chim. Acta.*, 936 (2016) 202.
- [20] Yang, G., Song, C., Shi, Q., Liu, H., Li, S., Liu, R., and Lv, C., *J. Pharm. Biomed. Anal.*, 159 (2018) 459.
- [21] Li, S., Liu, H., Yang, G., Liu, S., Liu, R., and Lv, C., *J. Environ. Radioact.*, 195 (2018) 60.
- [22] Liu, H., Chen, Y., Song, C., Tian, G., Li, S., Yang, G., and Lv, C., *Anal. Bioanal. Chem.*, 410 (17) (2018) 4227.