Bacterial Bio-detector for $CdCl_2$ and $NiCl_2$ Heavy Metal Pollutants Based on Their Optical Properties

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ABSTRACT

In fundamental neuroscience and cell biology, gadgets for studying living cells have been of great interest. Recent research has expanded cell-based sensors to pharmacological screening, environmental monitoring, and toxicological detection. Among the many measurement methods, optical sensors make bioactivity and cell population analysis easy. This research is part of a larger effort to develop microorganism-based heavy metal (toxin) sensing methods. A correlation was established to examine how heavy metals affect E. Coli and Deinococcus radiodurans bacteria's optical density. CdCl2 and NiCl2 were used for this study. In this study, Escherichia coli (E. coli) and Deinococcus radiodurans (D. radiodurans) were exposed to heavy metal solutions. Dissolving the compounds in de-ionized water generated 0.1, 1, 10, 100, and 1 M CdCl2 and NiCl2. This study used fluorescence microscopy, spectrophotometers, and fluorescence spectroscopy.

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1. Introduction

Thousands of sites around the world contain many types of pollutants. Generally, pollution is harmful to all lives on this planet due to; direct effect through the damage of critical part of cell for example: DNA, RNA and Protein so the cells at high level of pollution its imminent death, also, the indirect effect through the damage of the cell chromosome followed by cells division and generation of cancer, or Pollution causes change in genetic characterization then a genetic mutations causing congenital malformation. Many biological parameters and processes can be sensed and monitored with optical bio-cell sensors, with the advantage of being a non-invasive and relatively cheap technique [1]. Furthermore, the optical characteristics as indicators for the cell growth and activity, changes in cell composition and shape or in cell counts are examples of how processes can be detect with optical-cell sensors. The main idea of this project is summarized to fabricate and develop a bio-cell sensor to detect the environmental pollution, the project was utilized bacteria for detection of Heavy Metals ($CdCl_2$ & $NiCl_2$), firstly, through developed the optical sensing technologies for detection (monitoring) of the environmental pollution [2], due to measuring and studying the optical properties for bacteria growth solution, which including both of broth medium and bacteria. E. coli and D. radiodurans bacteria was utilized in this project for this task. The change in bacteria count was dependent as indicator to estimate the magnitude of heavy metals concentrations, secondly, studied the ability of bacteria to resist the environmental contaminants (through tested or measured the bacterial counts (density) changing after exposed to pollutants. Recently this technique is considered efficient method to monitor the environmental pollution levels. This method may give good results and useful information required for basic considerations. This method is inexpensive, fast, easy to implement and interpret, and provides the necessary information with a certain accuracy.

2. Experimental

2.1. Bacteria sample preparation

The most common bacteria Escherichia coli (or shortly, E. coli) and Deinococcus Radiodurans (D. radiodurans) were employed in this work as sensitive materials to detect the heavy metals pollution. Two species of bacteria: Gram-negative bacteria Escherichia coli (E. coli) and Gram-positive bacteria Deinococcus Radiodurans (D. Radiodurans). The non-pathogenic HD5α strain of

Escherichia coli in LB broth (Luria-Bertain) was used as a medium for culture of Escherichia coli cells. Anderson R1 strain of D. Radiodurans, which is highly resistant to ionizing radiation, UV rays, dehydration, oxidizing and electrophonic agents were used in this work with nutrient agar (oxoid cm^3). Both types of bacteria and related growth media were obtained from SIGMA-ALDRICH CO. and OXOID LTD. Other chemicals, such as $CdCl_2$, $NiCl_2$ a salts were purchased from SIGMA-ALDRICH CO. Bacterial culture was carried out in several stages. The first step was to grow a specific strain of bacteria in a Petri dish containing solid broth agar. In the second stage, a single colony of bacteria was added to a sterile beaker containing 50 ml of liquid broth. Finally, the vial containing the bacterial culture was placed inside a shaking incubator operating at a shaking speed of 150 rpm. Incubation temperatures were 30 °C for D. Radiodurans and 37 °C for Escherichia coli. Bacteria begin to grow 16 hours later for Escherichia coli and 24 hours for D. Radiodurans [3].

2.2. Experimental procedures

Three different optical techniques: (i) fluorescence microscopy that directly produces the ratio of live/dead bacteria, stained, respectively, with “green” and “red” fluorescent dyes, (ii) optical density measurements at 600 nm, and (iii) fluorescent spectroscopy were used. The results obtained were encouraging; all three optical methods give consistent and correlated results in regards to the heavy metals. Solutions of different concentrations of $CdCl_2$, $NiCl_2$ (down 0.1mM) were prepared by multiple dilution of 1M stock solution of metals in de-ionised water. Bacteria samples were mixed with each metal individually solutions in 1:1 ratio and kept incubated from 1 hour until 384 hours. As mentioned earlier there are two main types of bacteria (gram-negative bacteria E. coli and gram-positive bacteria D. radiodurans) [4].

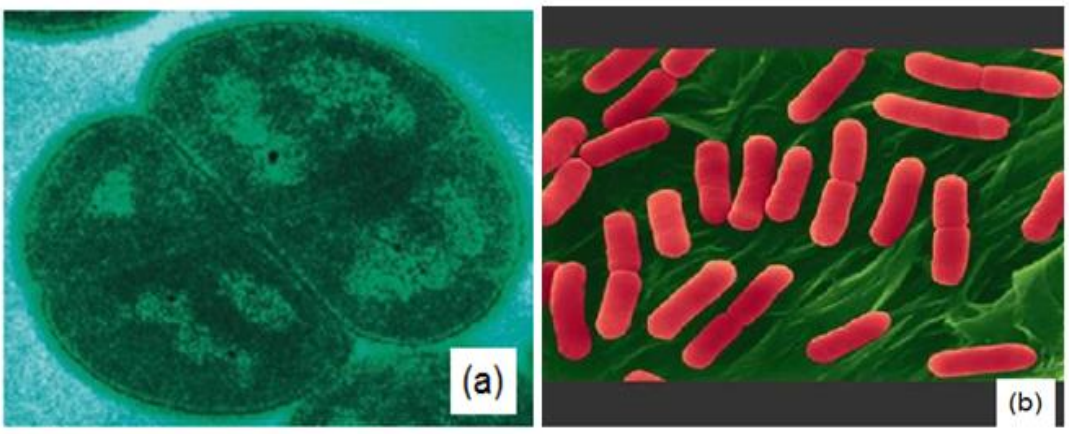

Fig 1: (1-a) gram positive bacteria cocci shape, (1-b) gram negative bacteria bacilli shape [4].

3. Results and discussion

The initial method employed is fluorescence microscopy, namely the Olympus-BX61 model. A Fluorescence Microscope was employed to assess the viability of bacteria, followed by staining the bacterial samples with SYTO-9, a green fluorescence nucleic acid stain. The presence of living bacteria exhibiting intact cell membranes is characterized by the emission of green fluorescence. Additionally, the utilization of propidium iodide, a red fluorescence nucleic acid stain, allows for the visualization of bacterial nucleic acids. The red fluorescence observed in dead bacteria with damaged membranes was a result of staining. A slide containing stained bacteria was subjected to illumination using blue light with a wavelength of 420nm [5]. In order to investigate the impact of heavy metals, the salts CdCl2 and NiCl2 were chosen [6]. Two distinct strains of bacteria were employed and afterwards combined with a solution containing heavy metals. Various quantities (0.1 mM, 1 mM, 10 mM, 100 mM, and 1 M) of (CdCl2 and NiCl2) were produced by dissolving the compounds in de-ionized water. The figure presented below depicts fluorescence microscopy pictures of samples containing E. coli bacteria. Panel (a) represents the sample without the addition of CdCl2, while panel (b) illustrates the sample with the inclusion of CdCl2, following a 72-hour incubation period. The impact of CdCl2 on the density of D. radiodurans bacteria was investigated and evaluated through the utilization of fluorescence microscopy [7]. The fluorescence microscopy approach was utilized to investigate the impact of heavy metals (namely NiCl2) on bacterial organisms, employing the same methodology as previously described.

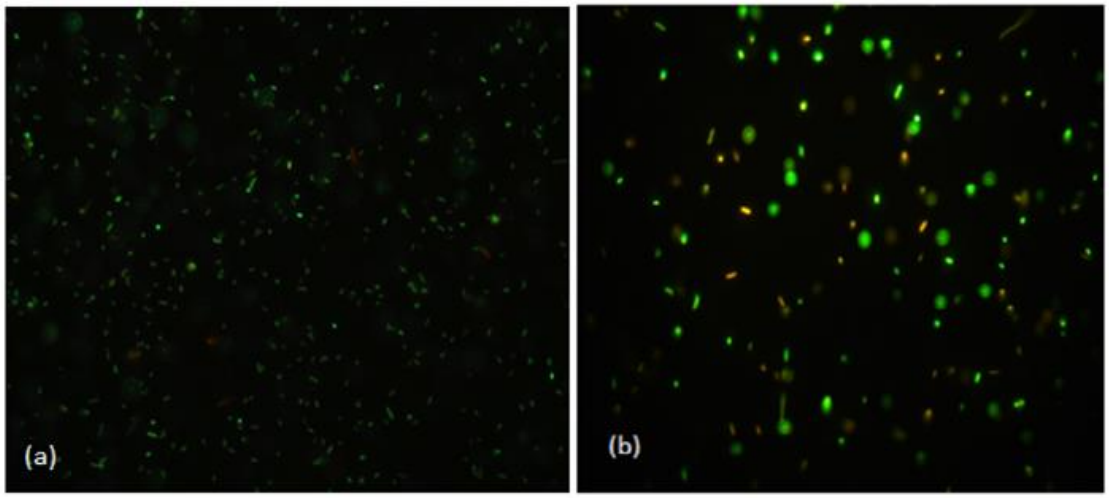

Fig. 2: Fluorescence microscopy images of E. coli bacteria samples (a) without, and (b) with $CdCl_2$.

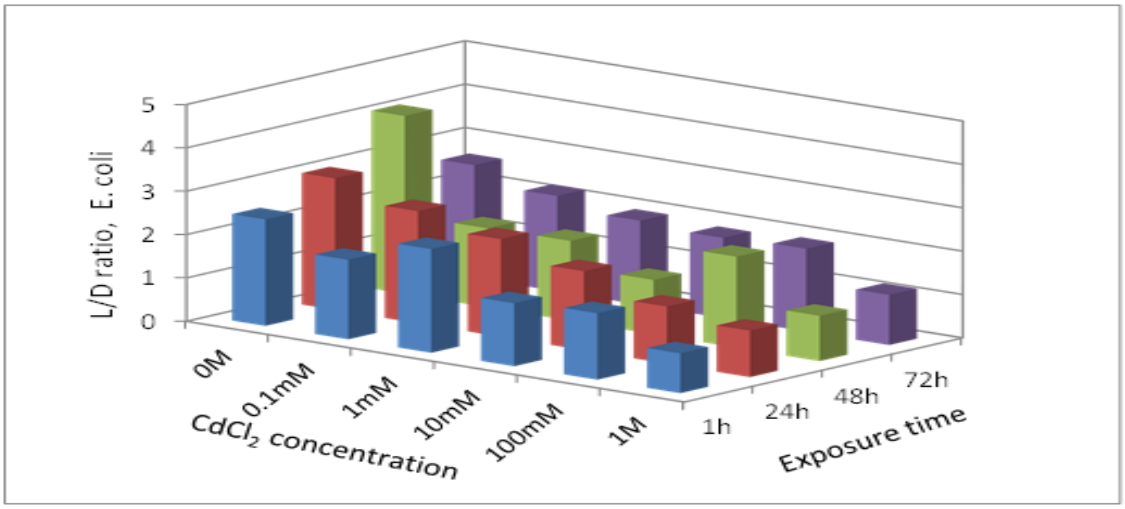

Fig. 3: Effect of $CdCl_2$ on the (Live/Died) ratio of E. coli bacteria for different time incubations.

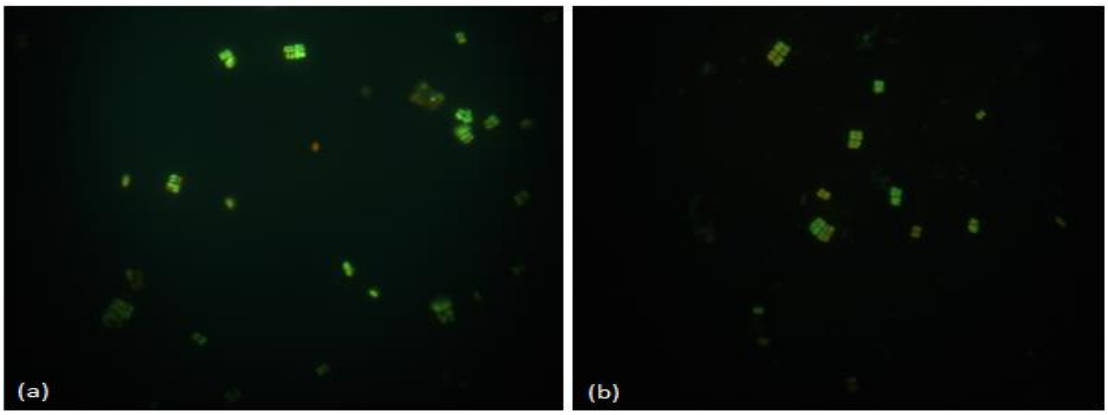

Fig. 4: Fluorescence microscopy images of D. radiodurans bacteria sample (a) without salt, and (b) with $CdCl_2$ salt after 550 hours adding the metal.

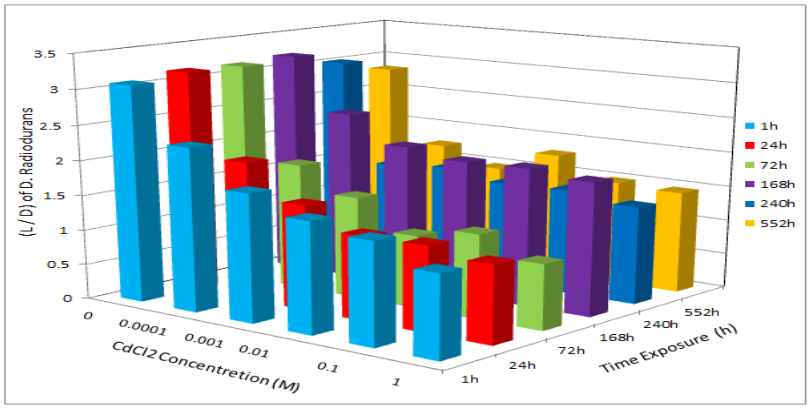

Fig.5: Effect of CdCl2 on L/D ratio of D. radiodurans for different incubation times.

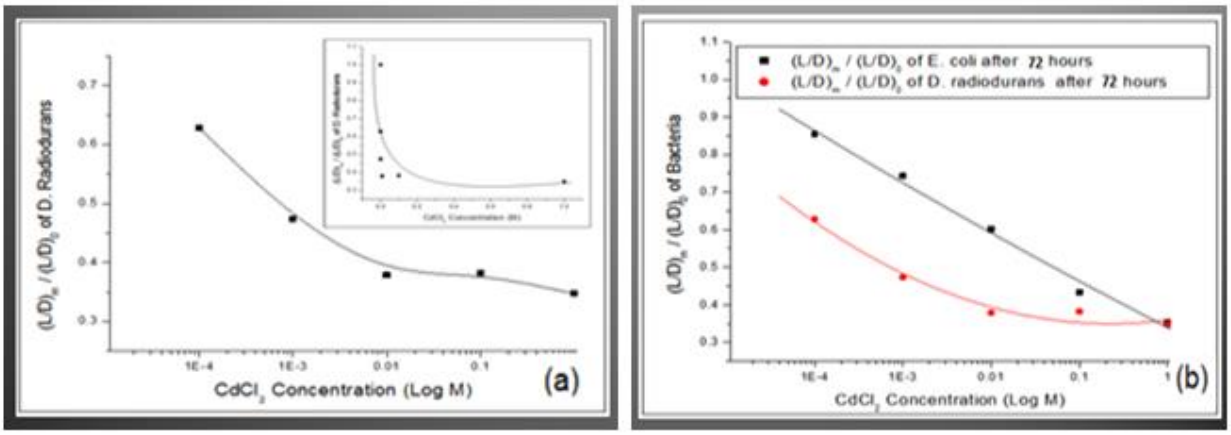

Fig 6: a- Ratio (L/D)m of D. radiodurans after adding salt over ratio $(L/D)_0$ of D. radiodurans without CdCl2, after 72 hours, b- Dependence of $((L/D)_m/ (L/D)_0)$ bacteria ratio for both E. coli and D. radiodurans bacteria treated with $CdCl_2$ (fluorescence microscopy results.

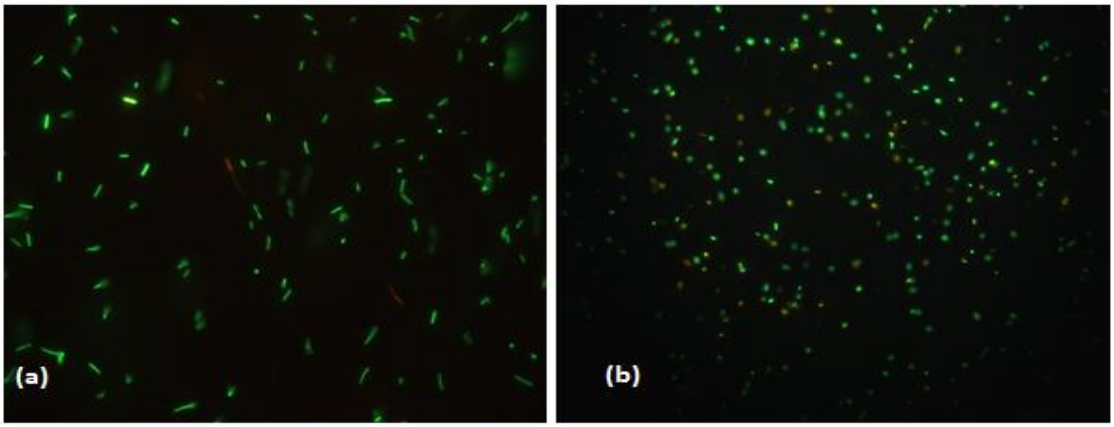

Fig.7: Fluorescence microscopy images of E. coli bacteria sample (a) without metal, (b) with $NiCl_2$ after 72 hours.

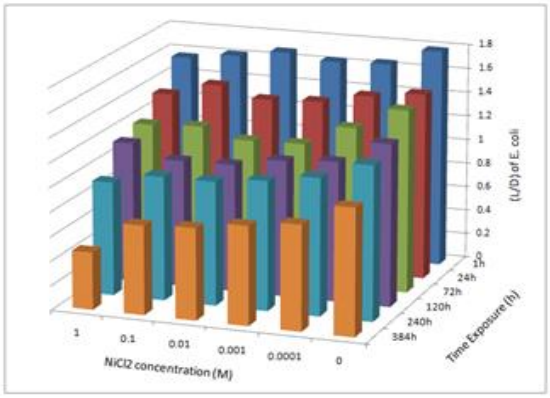

Fig. 8: Effect of $NiCl_2$ on (L/D) ratio of E. coli salt and time incubations against ratio $(L/D)_0$

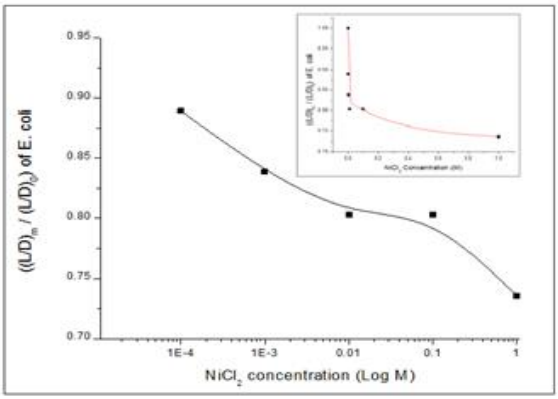

Fig. 9: Ratio $(L/D)_m$ of E. coli after adding for $NiCl_2$, after 72 hours exposure

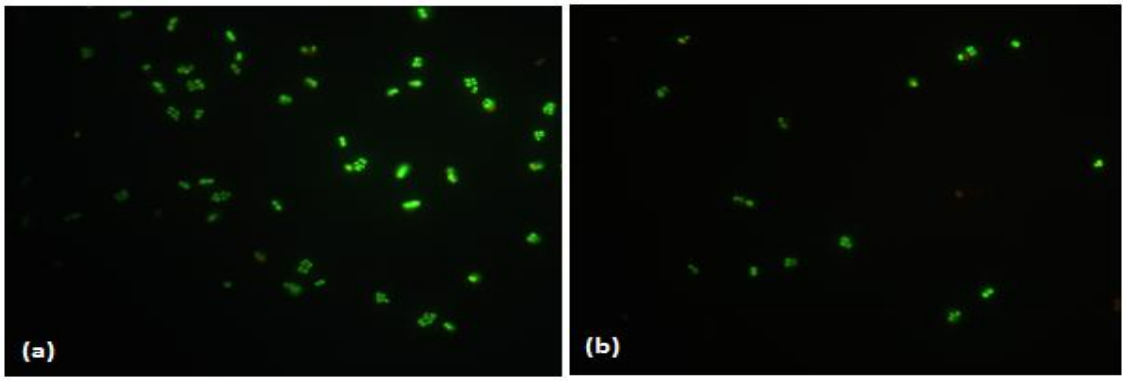

Fig. 10: Fluorescence microscopy images of D. radiodurans bacteria sample (a) without metal, (b) with $NiCl_2$ after 120 hours.

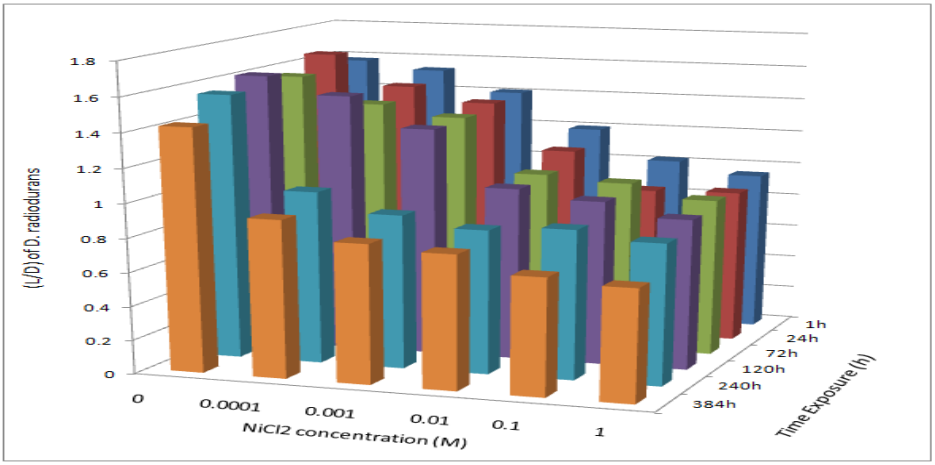

Fig.11: Effect of $NiCl_2$ on L/D ratio of D. radiodurans for different time incubations.

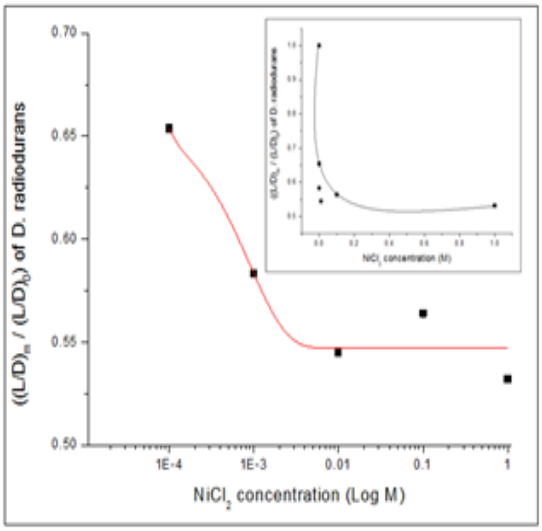

Fig.12: Ratio $(L/D)_m$ of D. radiodurans after adding $NiCl_2$ adding $NiCl_2$ over the ratio $(L/D)_0$ of D. radiodurans without $NiCl_2$, after 72 hours

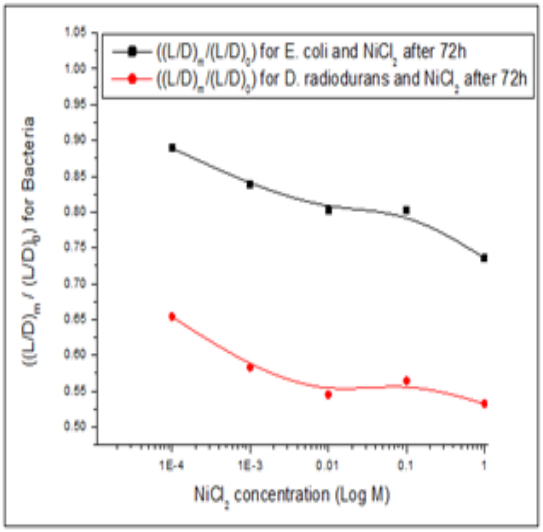

Fig 13: Dependence of $(L/D)_m/ (L/D)_0$ bacteria ratio for both E. coli and D. radiodurans bacteria for 72 hours exposure time to $NiCl_2$

Spectrophotometers Technique bacterial density measurement, an optical density (OD_{600}) technique was also used to estimate the bacteria cells density as a function of $CdCl_2$ concentration and the time exposed to metals [9].

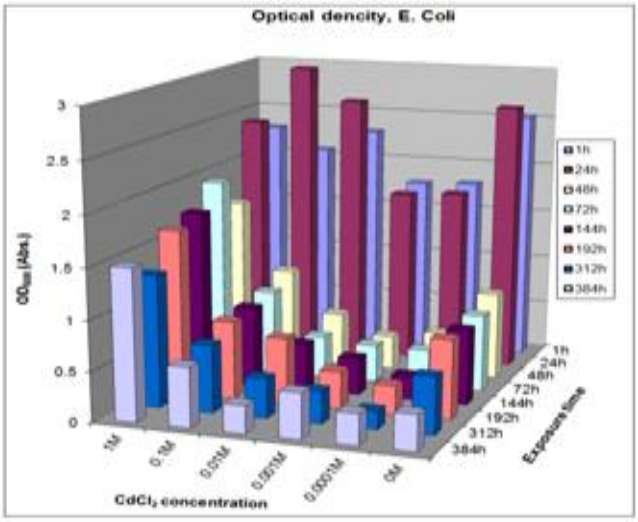

Fig. 14: The Optical Density test: optical densities OD_{600} for E. coli bacteria versus $CdCl_2$ concentration and time exposure

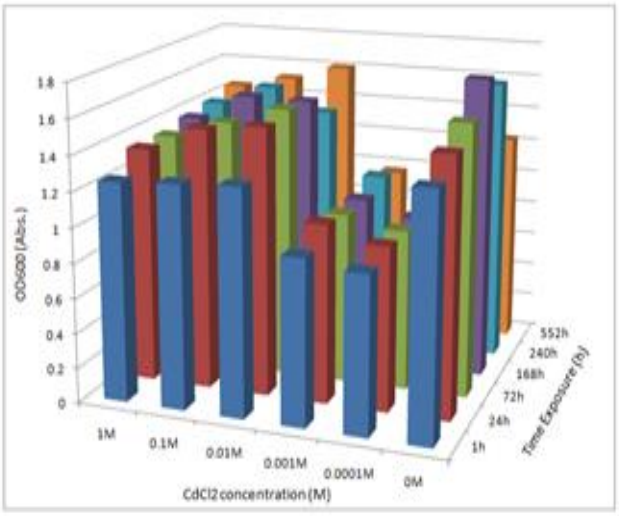

Fig.15: Optical densities OD_{600} for D. radiodurans bacteria versus time exposure to $CdCl_2$ for different times in the shake

The E. coli bacterial strain exhibited a notable sensitivity to cadmium chloride, with the manifestation of a metallic response observed within a mere two-day period of exposure, even at minimal concentrations. In contrast, D. radiodurans exhibited a notable level of resistance to the metal cadmium chloride. Furthermore, the optical density at 600 nm (OD600) measurements were also documented in relation to the concentration of NiCl2 and the duration of exposure to metals for both E. coli and D. radiodurans bacterial strains.

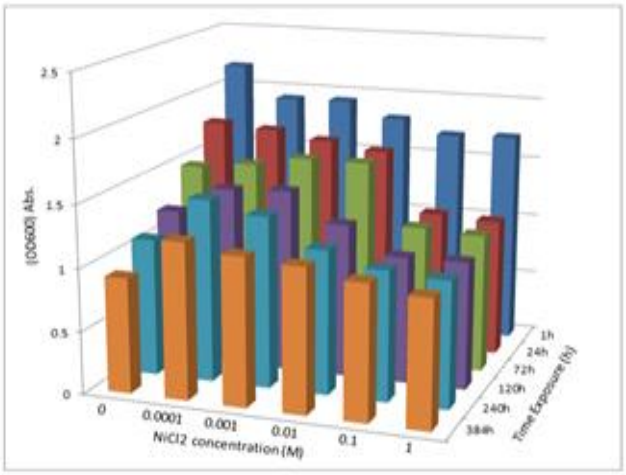

Fig. 16: Optical densities OD_{600} for E. coli bacteria versus $NiCl_2$ concentration and time exposure

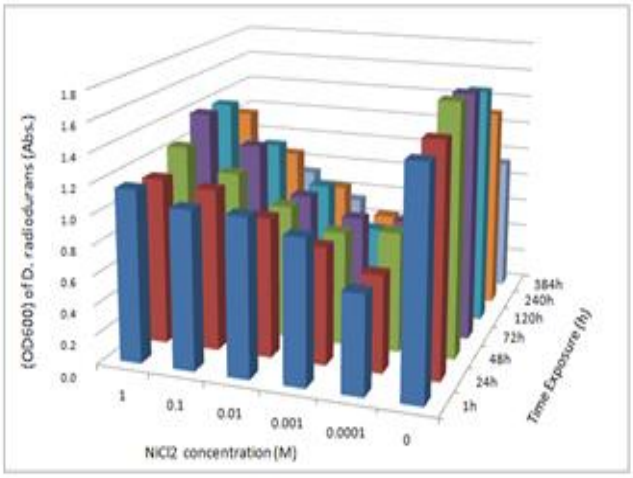

Fig. 17: Optical densities OD_{600} for D. radiodurans bacteria versus the $NiCl_2$ concentration and time exposure

Figures 16 and 17 provide evidence indicating that the two bacterial strains exhibit a modest response when subjected to nickel chloride metal, even under conditions of elevated concentrations and prolonged exposure. The fluorescence spectroscopy technique involves the utilization of a laser beam to stimulate the electrons within certain compound molecules, hence inducing the emission of light. The light is directed towards a filter and afterwards onto a detector in order to quantify and characterize the molecule or any alterations occurring within the molecule. In order to validate the results mentioned above about the impact of $CdCl_2$ and $NiCl_2$ on the bacteria E. coli and D. radiodurans, the utilization of fluorescence spectroscopy was investigated [8].

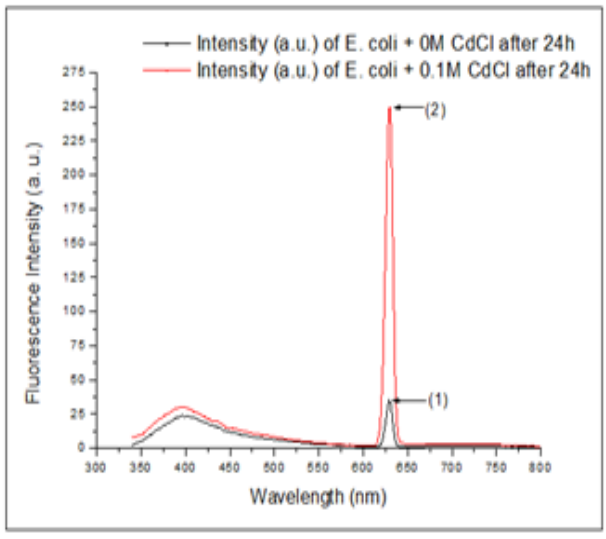

Fig 18: Fluorescence spectra of two E. coli Bacteria samples: (1) no $CdCl_2$; (2) mixed with $CdCl_2$ for 24h

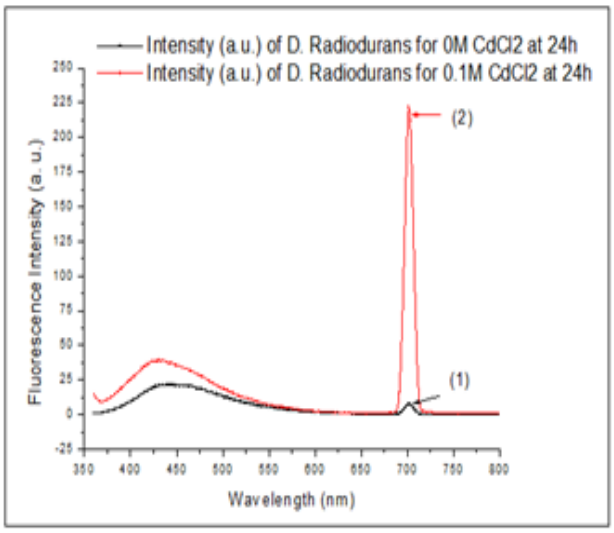

Fig. 19: Fluorescence spectra of two D. radiodurans bacteria samples: (1) no $CdCl_2$; and (2) mixed with $CdCl_2$ for 24h

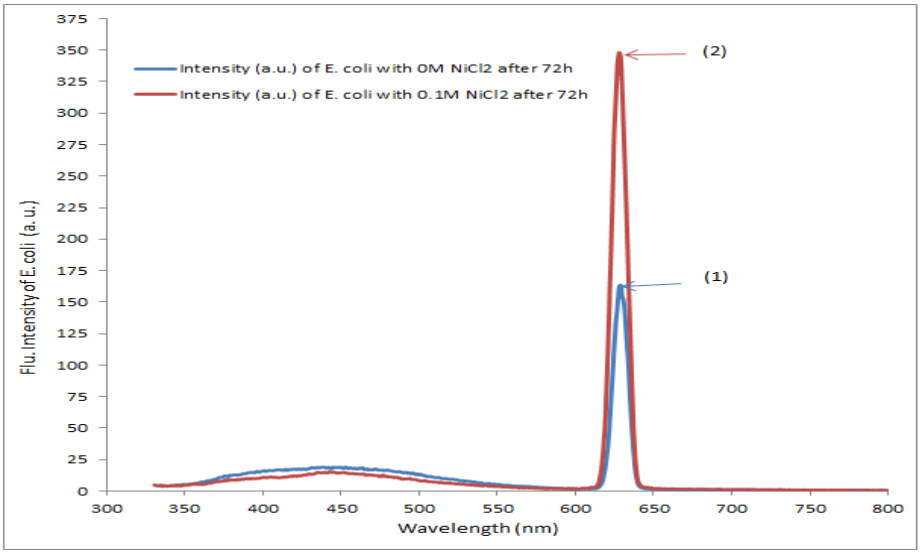

Fig. 20: Fluorescence spectra of two E. coli bacteria samples: (1) not mixed with $NiCl_2$; and (2) mixed with (0.1 mol) of $NiCl_2$ after 72h.

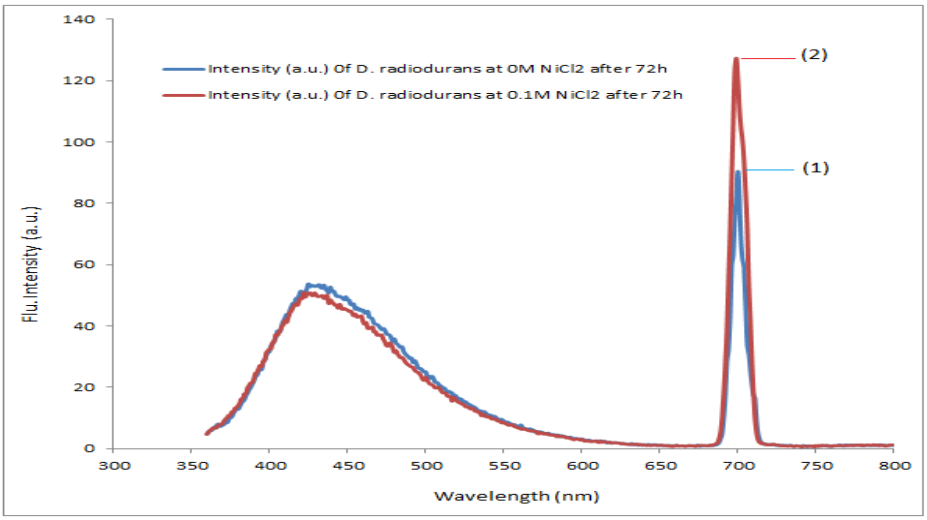

Fig. 21: Fluorescence spectra of two D. radiodurans bacteria samples: (1) not mixed with $NiCl_2$ and (2) mixed with (0.1 mol) of $NiCl_2$ after 72h.

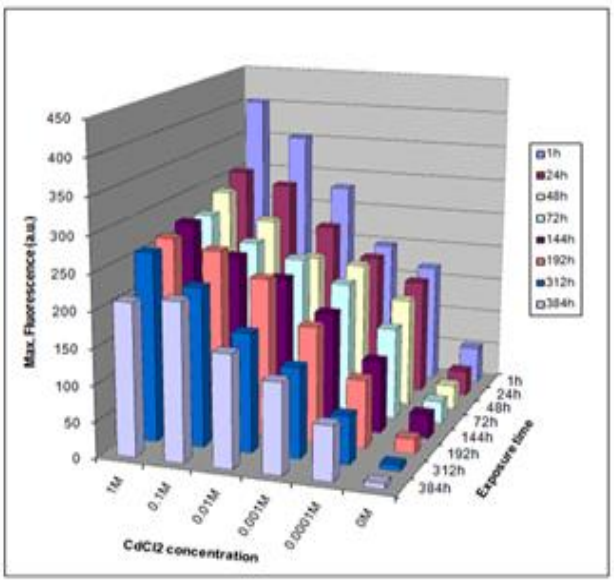

Fig. 22: Effect of $CdCl_2$ on 2-nd order diffraction peak for E. coli, fluorescence for bacteria sample exposed to $CdCl_2$

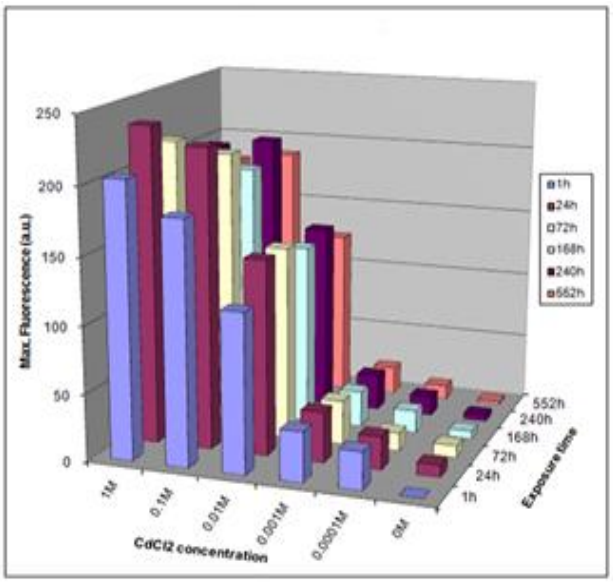

Fig. 23: Effect of $CdCl_2$ on 2-nd order diffraction peak for D. radiodurans, fluo for bacteria sample exposed to $CdCl_2$

Figure 24 shows the two type bacteria response for $CdCl_2$, the curve showing the metal catalyzed D. radiodurans bacteria to grow up, whilst the E. coli bacteria clearly unaffected.

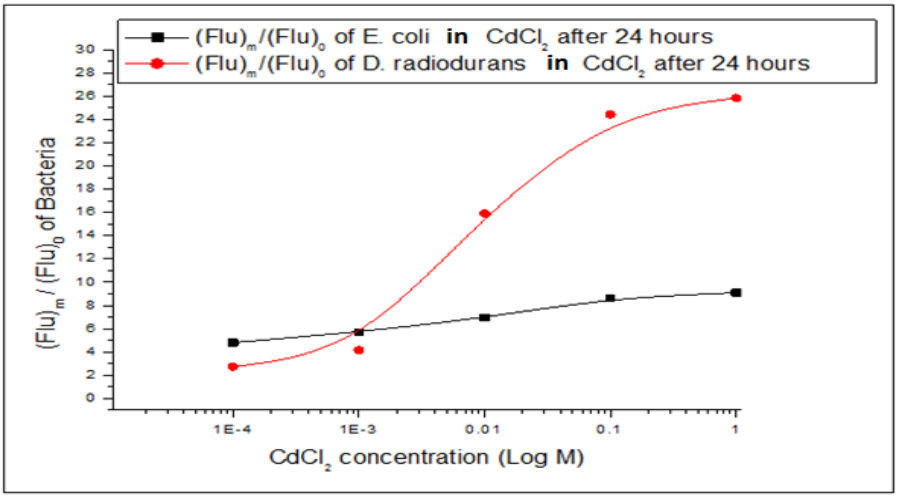

Fig. 24: Effect of $CdCl_2$ on 2-nd order diffraction peak for E. coli (black) and D. radiodurans (red) bacteria.

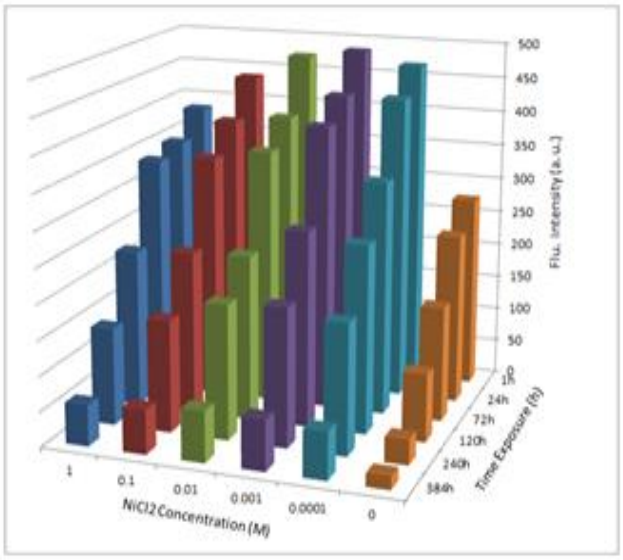

Fig.25: Effect of $NiCl_2$ on second order diffraction for E. coli bacteria sample

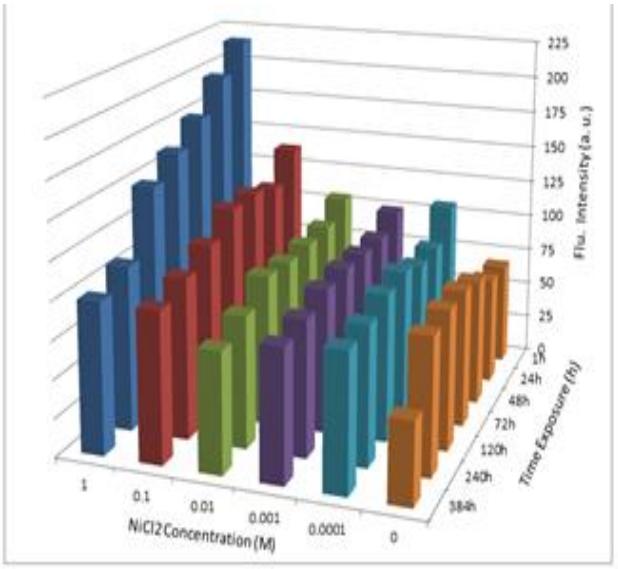

Fig.26: Effect of $NiCl_2$ on second order diffraction peak for D. radiodurans bacteria

The effect of heavy metals on bacteria were studied during was examined, the figures 27 and 28 showing the two type of bacteria response for that heavy metals.

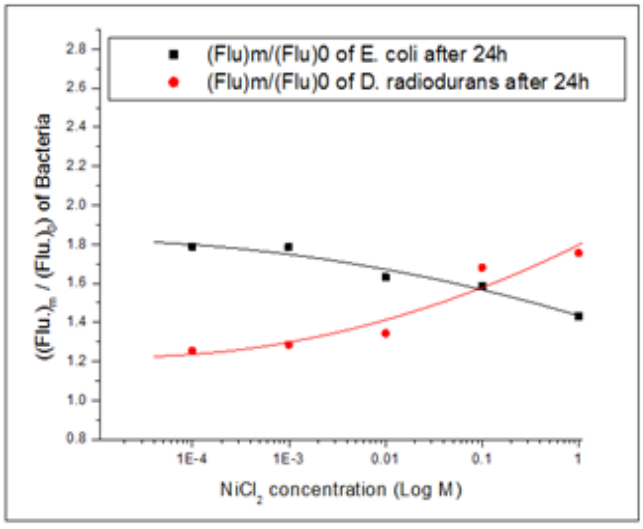

Fig. 27: Effect of $NiCl_2$ on 2-nd order diffraction peak for E. coli and D. radiodurans bacteria

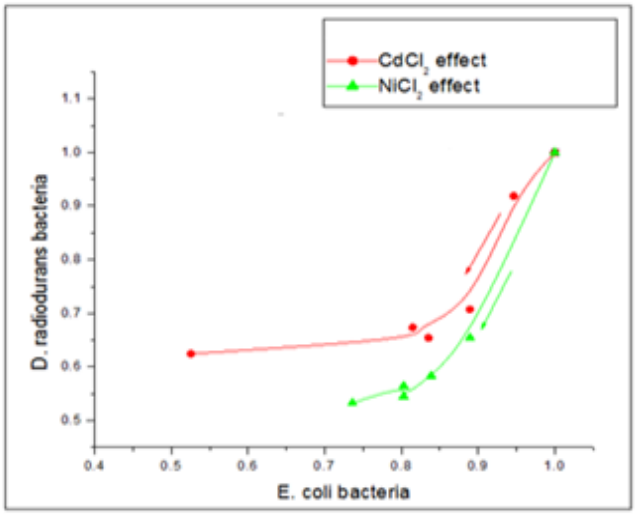

Fig. 28: Comparisons of relative changes in (L/D) ratio, for E. coli and D. radiodurans bacteria in response to

The points at the double excitation wavelength, which presented a second-order diffraction peak that correlates with the bacteria density in the samples, these results were shown in figure 27. From this point of view, the intensity of the2-order diffraction peak increases directly when the live bacteria concentration increases and verse versa. Figure 28 shows the pattern recognition of the effect of $CdCl_2$ and $NiCl_2$ on bacteria samples; from the graphs, you can see the response of E. coli bacteria that seem to be very sensitive to heavy metals, Otherwise, D. radiodurans bacteria showed an opposite response to that of E. coli bacteria.

Conclusions

Using the optical properties of the biocells sensor, numerous biological parameters and metabolic processes can be studied and tracked. Optical methods including (optical density measurements, UV-vis spectrophotometer, fluorescent microscopy and spectroscopy) were used to construct a heavy metals biosensor using a variety of experimental techniques, namely optical methods. In terms of (Abs) absorption rate, the correlation between E. coli and D. radiodurans bacteria concentration and light density intensity (OD600) was established. The number of bacteria in the study sample is (Cell/ml = Abs * 8 * 10^8) if the broth was used as the growth medium and was regarded a control. $CdCl_2$ appeared to have a similar effect on E. coli and D. radiodurans bacteria, but NiCl2 appeared to have a distinct effect on the bacteria samples. Fluorescence microscopy appears to provide the most accurate estimate of active bacteria concentration. The fluorescence microscopy data showed that the number of live D. radiodurans bacteria gradually increased as the concentration of $CdCl_2$ was increased (figure 24), while the E. coli bacteria type demonstrated the opposite response to $CdCl_2$ (figure 27). We can use these two varieties of bacteria to construct and form a biosensor that can detect the presence of the heavy metals $CdCl_2$ and $NiCl_2$ based on what has been discussed previously.

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أعتماد الخواص الضوئية للبكتريا كأساس لكاشف حيوي عن الملوثات المعدنية الثقيلة ($CdCl_2$, $NiCl_2$)

ميثم عبدالله علي

قسم الفيزياء – كلية العلوم – جامعة البصرة

المستخلص

الأجهزة المتخصصة في دراسة سلوك الخلايا الحية تلقى اهتمام كبير كونها توفر معلومات دقيقة عن الخلاية الحيوية المختلفة. هذا البحث تضمن دراسة موسعة للخواص الضوئية للخلايا الحية التي استخدمت كاساس لعمل متحسس في طيف واسع من التطبيقات الصيدلانية والبيئية وكذالك للكشف عن السموم. بالأضافة الى ذالك تم اجراء قياسات بتقنيات مختلفة, المتحسسات الحيوية الضوئية تعطي مؤشرات واضحة ومبسطة لمراقبة النشاط الحيوي وكذالك تدرس خواص المجمعات الخلايا البكتيرية البحث يمثل جزء من عمل متخصص في تطوير متحسسات مبتكرة تستخدم كتقنية للكشف عن المعادن الثقيلة (السامة) باستخدام الأحياء المجهرية. تمت دراسة وبحث العلاقة بين الخواص الضوئية (الكثافة الضوئية) للخلايا وتركيز الخلايا البكتيرية للبكتريا المعوية E Coli وكذالك بكتريا Deinococcus radiodurans, تمت دراسة تأثير المعادن الثقيلة كأملاح ثاني كلوريد الكادميوم وكذالك ثاني كلوريد النيكل على الخلايا الحية من خلال دراسة الخواص الضوئية لها.تم أختيار نوعين من البكتريا الغير ضارة (friendly type) هما E Coli وكذالك Deinococcus radiodurans حيث تم مزج المحاليل الملحية الحاوية على المعادن الثقيلة وبتراكيز مختلفة هي (0.1 ملي مول, 1 ملي مول, 10 ملي مول, 100 ملي مول, وكذالك 1 مول) حيث حضرت بماء منزوع الأيونات. عدة تقنيات ضوئية تم استخدامها وهي: - مجهر التألق الضوئي, مقياس الضوئي الطيفي لدراسة كثافة الخلايا البكتيرية ضوئيا, ومقياس التألق الطيفي. أضهرت النتائج استجابة واضحة ومتباينة للبكتريا لتراكيز المختلفة للمعادن الثقيلة, مما يشجع في استخدام هذه الأنواع من البكتريا كمادة فعالة في عمل وبناء متحسس حيوي ضوئي للمعادن انفة الذكر, وقد ادرجت هذه الأستجابات البكتيرية بشكل تفصيلي من خلال النتائج التي تضمنها متن البحث.