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Biosensors for Heavy Metal Detection in Environment: A review

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Abstract

Heavy metals are major environment pollutants due to their extreme toxicity, non-biodegradability and bioaccumulation properties that they exert in plants, animals and humans. Heavy metals act as inhibitors of enzymes, which, are unable to bind the substrate. There is need for the fast and inexpensive methods for the detection of heavy metals. Biosensors are useful devices in this area. A biosensor is an analytical device, which converts a biological response into an electrical signal. The biosensor consists of two main components; bio receptor and transducer. Bio-component like enzymes, metalloproteins or microbes are used to recognizes the target molecule. Conventional techniques to analyse metals include cold vapour atomic absorption spectrometry, inductively coupled plasma mass spectrometry, UV visible spectrophotometry and X-ray absorption spectroscopy. The mechanism of enzyme-based biosensor is reviewed. The bioelectronics, nanotechnology, and bioengineering will compete for developing sensitive and selective biosensors able to determine multiple analytes simultaneously in environmental matrix. The overall objective of this review is to present information concerning sources of heavy metals and different mechanisms of Biosensors for the detection of heavy metals.

Keywords: Heavy metals, enzymes, bio-component, pollutants, Biosensors

Introduction

Heavy metals are major environment pollutants due to their extreme toxicity, non-biodegradability and bioaccumulation properties that they exert in plants, animals and humans (Mates et al., 2010). The presence of heavy metals in excess affects air, water as well as soil. The plants grown in such areas can accumulate heavy metals like cadmium, zinc, lead, chromium, mercury and copper. Many of these metals are carcinogens that have been closely linked to several diseases such as Alzheimer's, Parkinson's, multiple sclerosis, osteoporosis, developmental disorders, and organ failures (Mates et al., 2010; Jomova and valko, 2011). Therefore, the detection of heavy metals is very important, particularly in the sources of environmental, as their mixture with other chemicals may exhibit complex toxicities. Conventional techniques to analyse metals include cold vapour atomic absorption spectrometry, inductively coupled plasma mass spectrometry, UV visible spectrophotometry and X-ray absorption spectroscopy. These techniques, although highly precise, suffer from the disadvantages of high cost, the need for trained personnel and the fact that they are mostly laboratory bound. For the reasons cited earlier, biosensors are now being utilized for monitoring heavy metal concentrations. Furthermore, their biological base makes them ideal for toxicological measurement of heavy metals, while conventional techniques can only measure concentration (Neelam Verma and Minni Singh, 2005). Many bioassays have been developed using algae, luminescence bacteria, plant tissues and animal cells, especially algal bioassay which is one of the popular toxicity tests.

Natural sources

In nature excessive levels of trace metals may occur by geographical phenomena like volcanic eruptions, weathering of rocks (Acid rock drainage) and leaching into rivers, lakes and oceans due to action of winds.

Anthropogenic Sources

In modern times, anthropogenic sources of heavy metals, i.e. pollution, have been introduced to the ecosystem. People have always been exposed to heavy metals in the environment. Metals leaching from eating utensils and cookware lead to metallic contamination of food and water. Metallic constituents of pesticides and therapeutic agents are additional sources of hazardous exposure. The burning of fossil fuels containing heavy metals, the addition of tetra-ethyl lead to gasoline, and the increase in industrial applications of metals, such as metal plating factories, mining industries, tanning, dye and chemical manufacturing industries, etc., have made heavy metal poisoning a major source of environmental pollution (Abdou KA, Khadiga IA 2016).

Determination of Heavy metal

Since the enzymatic activity is inhibited by the presence of heavy metals, the concentration of these chemicals can be assessed by measuring the enzymatic activity. Over the past three decades, several techniques using the various enzymes have been proposed: oxydases and dehydrogenases, and alkaline phosphatase. In addition, some studies have been reported about utilization of the enzymatic activity inhibition of algae by heavy metals. In these researches, they are using several enzymes in algae, such as alkaline phosphatase, acetylcholinesterase, and esterase. The enzyme inhibition in microalgae is becoming a well-accepted indicator of environmental stress because of the rapid and sensitive endpoint.

Materials and Methods

Biosensor is a synergistic combination of biotechnology and microelectronics. Basically biosensor is an analytical device that consists of an immobilized biological material in intimate contact with a compatible transducer, which will convert the biochemical signal into a quantifiable electrical signal (Gronow 1984). The biomolecules are responsible for the specific recognition of the analyte whereas the physicochemical transducer supplies an electrical output signal which is amplified by the electronic component.

Enzymes are used in biosensors because of their specificity. Most of the enzymes used in sensors have been isolated from microorganisms; therefore, it is logical that the organisms themselves should be regarded as potential biocatalysts. In microorganisms, the enzymes remain in their natural environment,

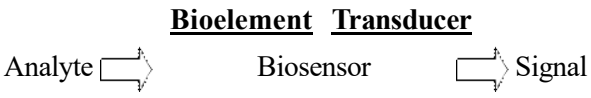

Fig 1: Schematic representation of a biosensor

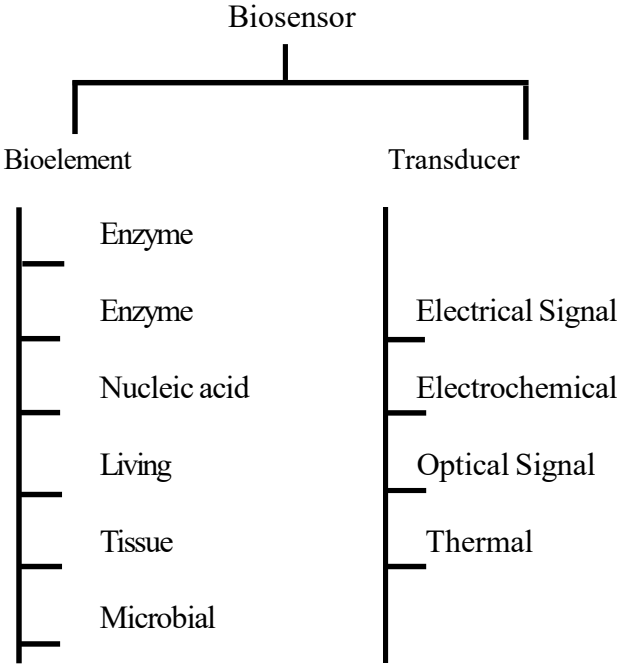

Fig 2: Examples of elements in biosensor

increasing stability and activity (D'Souza, 2001; Verma and Singh, 2003). Cell membranes and organelles can also be used for biosensor construction (Verma and Malaku, 2001). Specific binding between antibody and antigen can be exploited in immunobiosensors. To detect very low concentrations of substances such as drugs, toxins or explosives, receptor-based sensors are very appealing (Prasad et al., 2004). Researchers analysed alkaline phosphatase by electrochemical sensing for diagnosis of various diseases (Zhang et al., 2013). ALP / para-nitrophenyl phosphate based biosensors using screen printed carbon electrode modified with gold nanoparticles has been also used for selective chronoamperometric determination of vanadium with a limit of detection of 0.39 µM.

Various immobilization procedures have been used in biosensor construction. In general, the choice of procedure depends on the nature of the biological element, the type of transducer used, physicochemical properties of the analyse and the operating conditions

Table 1: Principle characteristics of enzyme biosensors for heavy metal detection (Turdean, 2011)

Enzyme	Detection; working electrode; applied potential	Limit of detection
AChE	Amperometry; G/AChE; 0.80 V versus SCE	10"10MHg2+
ALP	Conductometry; Au/ALP-GA-BSA-glycerol	0.5 ppm Cd2+, 2 ppm Zn2+,2 ppm Co2+, 5 ppm Ni2+,40 ppm Pb2+
ALP	Amperometry; SPE/ALP-chitosan; +0.6 versus Ag/AgCl	mg/L Hg2+, Cd2+, Ag+,Zn2+, Cu2+
ALP	Fluorescence	10.1 *i*MAg+
ALP	Chemiluminescence	0.36 ppb Zn2+
Gox	Amperometry; Pt/PA/Fc/GA/GOx; +0.7V versus SCE	0.49 *i*g/LHg2+
Inv Gox	Amperometry; Pt/Inv-Gox-agarose-guar gum; + 0.35 versus Ag/AgCl	5″10″10MHg2+, 3″10″8MPb2+, 5″10″8MAg+, 2.5″10″8MCd2+
Inv Mu Gox	Amperometry; Pt/Inv-Mu-Gox-laponite; +0.6 versusAg/AgCl	3ppbHg2+
NR	Conductometry; Pt/NR–GA-BSA-metyl viologen/Nafion	0.05 *i*MCu 2+, 0.5*i*MZn2+, 0.1*i*MCd2+
PQQ-GDH	Amperometry; G/PQQ-GDH-GA; +0.25 versusAg/AgCl	15″10″5MCd2+, 15″10″5MPb2+
Ty	Amperometry; GCE/Ty-pPy;+0.4 versus Ag/AgCl	5 10″7MCr3+
Urease	Amperometry; Pt/PVF/urease;+0.7V versus SCE	7.4 *i*MHg2+
Urease	Potentiometry; PVC-NH2/Au-NP/urease	0.05 *i*MHg2+
Urease	Potentiometry; Ir/IrO2/Urease-PVC	0.02 *i*MHg2+
Urease	Conductometry; SPE	Cu2+, 0.1mMCd2+,0.9mM Pb2+
Urease/GLDH	Amperometry; C-Rh; +0.3 versus Ag/AgCl	7.2 *i*g/L Hg2+, 8.5*i*g/LCu2+, 0.3mg/L Cd2+,0.2mg/L Zn2+

ALP = alkaline phosphatase; Au-NP = gold nanoparticles; BSA = bovine serum albumin; GA = glutaraldehyde; GdA = glutaric dialdehyde; GCE= glassy carbon electrode; GLDH = glutamate dehydrogenase; Gox = glucose oxidase; Inv = invertase; Mu = mutarotase; NR = nitrate reductase; PQQ-GDH = pyrroloquinoline quinine dependent glucose dehydrogenase; PVC = poly(vinyl chloride); PVC-NH2 = ethylenediamine poly (vinyl chloride); PVF = poly (vinylferrocenium); SCE = saturated calomel electrode; SPE = screen-printed electrode; Ty = tyrosinase.

in which the biosensor have to function. Perhaps overriding all the considerations is the necessity for the biological component to exhibit high activity with appropriate specificity in its immobilized microenvironment. The four main approaches to enzyme and microbial immobilization are entrapment and encapsulation, covalent binding, cross linking and adsorption (Verma and Singh, 2005). Some of the major attributes of a good biosensing system are its specificity, reliability, portability, (in most cases) ability to function in optically opaque solutions, real-time analysis and simplicity of operation (D'Souza 2001).

Signal measurement techniques

Electrochemical techniques

Amperometry is operated at a given applied potential between the working electrode and the reference electrode and the current signal is recorded and correlated with the concentration of target compounds. In the amperometric detection, the current signal is generated due to the reduction or oxidation of an electro-active metabolic product or intermediate on the surface of a working electrode.

Conductometry is a technique depending on the conductivity change in the solution due to the production or consumption of ionic species, for example, by the metabolic activity of the microorganisms. The measurement of conductance is extremely fast and sensitive but the selectivity of conductometric biosensors is relatively poor.

Potentiometry involves the measurement of the potential difference between the working electrode and the reference electrode. The transducer employed in the potentiometric technique is usually a gas-sensing

electrode or an ion-selective electrode. The sensitivity and selectivity of potentiometric biosensor are outstanding due to the species-selective working electrode used in the system. However, a highly stable and accurate reference electrode is always required and challenging to maintain, which may potentially limit the application of potentiometry in microbial biosensors.

Voltammetry is the most versatile technique in electrochemical analysis. Both the current and the potential are measured and recorded. The position of peak current is related to the specific chemical and the peak current density is proportional to the concentration of the corresponding species. A remarkable advantage of voltammetry is the low noise which can endow the biosensor with higher sensitivity. In addition, voltammetry is able to detect multiple compounds, which have different peak potentials, in a single electrochemical experiment (or scan), thus offering the simultaneous detection of multiple analytes.

Optical detection is usually based on the measurement of luminescent, fluorescent, colorimetric, or other optical signals produced by the interaction of microorganisms with the analytes and correlates the observed optical signal with the concentration of target compounds. Optical sensing techniques are especially attractive in high throughput screening since they enable biosensors to monitor multiple analytes simultaneously.

Fluorescent sensing technique is based on the measurement of fluorescence intensity which is proportional to the concentration of the target analyte. Fluorescence can be detected at a longer wavelength after the excitation of the fluorescent substance at a shorter wavelength. Fluorescent biosensors have been widely applied in analytical chemistry due to their easy construction using standard molecular biology techniques.

Bioluminescent sensing technique used in microbial biosensors relies on detecting the change of luminescence emitted by living microorganisms which responds to the target analyte in a dose-dependent manner.

Colorimetric sensing technique in microbial biosensors involves the conversion of a chromogen substrate into a colorful compound by the metabolic activity of the microbial sensing element. The colored product can be distinguished by the naked eye or a spectrophotometer. Because of its simple and inexpensive measurement setup, colorimetric technique has been widely applied in the fabrication of cost-effective microbial biosensors.

Numerous enzymes have been utilized for heavy metal detection. Alkaline phosphatase is an enzyme of high research interest, having also a variety of industrial applications. In a previous research, the ALP of Penaeus merguiensis has been purified and characterized. They found that the sensitivity of the enzyme increases against the inhibitory effects ions of the first transition series that are elements in marine pollution. Such inhibition may be due to masking of binding sites on the enzyme by these metal ions. The experimental results showed that all these metal ions also inhibited the activity of ALP from Penaeus merguiensis. Therefore, marine pollution might disrupt the production of Penaeus merguiensis (Homaei, 2015, 2017). Biosensors based on the principle of enzyme inhibition have been applied for a wide range of significant analytes such as organo-phosphorous pesticides (OP), organo-chlorine pesticides, derivatives of insecticides and heavy metal ions (Van Dyk and Pletschke, 2011; Tekaya et al. 2013; Chouteau et al. 2004; Chouteau et al. 2005). An amperometric glucose biosensor based on glucose oxidase was successfully applied for determination of a wide group of heavy metals (Hg^{++} , Ag^{+}, Cu^{+1}, Cd^{+2}, Pb^{+2}, Cr^{+3}, Fe^{+3}, Co^{+2},Ni^{+2}, Zn^{+2}, Mn^{+2} and CrO_4^{-2} (Guascito *et al*, 2008). They used saturated calomel electrode as reference electrode. Employment of alkaline phosphatase for assessment of heavy metal toxicity appears to be simple and sensitive tool for the quantitation; a method basically based on the strong inhibitory effect of these metal ions on alkaline phosphatase activity. The aim of this study was to develop an electrochemical bioassay for the evaluation of heavy metals toxicity using alkaline phosphatase (ALP). Para-nitrophenyl phosphate (PNPP) was used as a substrate for the voltammetric determination of the alkaline phosphatase activity. The alkaline phosphatase converts the PNPP into p-nitrophenol (PNP), which can be oxidized to quinone forms via irreversible electrochemical reaction.

A sensitive electrochemical determination of ALP activity can be performed from the oxidation current of PNP by means of cyclic voltammetry using graphene oxide modified indium tin oxide (ITO) electrode. The electrochemical approach is better compared to conventional optical detection because it

does not require bulky equipment; it can be disposed easily, can be operated on-site and can be used for coloured samples also. Another important advantage of this technique is that it does not require much dilution. In recent years, biosensors are gaining importance as suitable detectors for heavy metal ions. They are very promising for environmental monitoring, due to simple, rapid and selective approach. Several techniques based on spectroscopy, ion-selective electrodes, polarography and voltametry have been described in the past. A urease based conductometric biosensor was developed by Zhylyak et al., 1995 for the heavy metal determination in wastewater. They immobilized enzyme by crosslinking it with bovine serum albumin, which forms a biologically sensitive membrane. An interdigitated gold electrode was used as the transducer and response of the sensor for various concentrations of heavy metal ions was evaluated by measuring the urease activity after incubating the electrodes in sample solutions of heavy metal ions. Li and co workers used horseradish peroxidise and developed an amperometric enzyme electrode for peroxide determination (Li et al., 1998). Urease isolated from pigeon pea was immobilized in poly acrylamide gel and calcium alginate beads analyzed for various performance factors. An enzyme-catalyzed polymer transformation when effectively combined with a transducer could be used effectively as a sensor (Ho et al., 1999). Some workers immobilized urease enzyme on gelatin beads via crosslinking with glutaraldehyde (Srivastava et al.,2001). The immobilized enzyme has been also analyzed for various performance factors by some workers (Ilangovan et al., 2006). Literature reports indicate that spectroscopic methods are expensive, as they require very sophisticated equipment, which cannot be used for field monitoring. But both polarographic and voltametric techniques lack selectivity. Since ion selective electrodes were based on the measurement of the potential at an electrode surface caused by a selective ion exchange reaction, the design of ion-selective membrane was a major difficulty in the development of this type of sensor. Taking into consideration the drawbacks mentioned above, there is a need for the development of a cheap, simple and portable detector for heavy metal ions. In many instances, monitoring is not continuous but requires a number of individual measurements to be made at different times. In such cases, sensors should be manufactured inexpensively so that they may be disposed after a single reading. Hence, the present work had the following objectives: To develop an enzyme-based biosensor, capable to detect heavy metal ions in environmetal matrix and to evaluate the performance of the sensor.

A calorimetric bio-sensing system for flow injection microanalysis of zinc ions has already been developed was based on alkaline phosphatase apoenzyme reactivation by the metal cofactor, a reaction that is exothermic. The enzyme was covalently immobilized and Zn (II) was detected over the range of 10 lM–1.0 mM with a response time of 3 min. The biosensor had a long-term operational stability of up to 2 months and was able to be regenerated in 2, 6-pryridine dicarboxylate solution (Satoh, 1991).

The more commonly heavy metal inhibition of enzymes is based, on the interaction of metal ions with exposed thiolor methylthiol-groups of protein amino acids. The inhibitory action of various metal ions on enzyme oxidases and dehydrogenases illustrate this behaviour for use in biosensors. The use of different enzymes in different buffer systems allowed measurements of specific heavy metals (Gayet et al. 1993). The inhibitory action of Cr has also been characterized using L-lactate dehydrogenase, hexokinase and pyruvate kinase. (Cowell et al. 1995). The inhibition of peroxidase by mercury has been studied after immobilization in chitosan films, on polystyrene plate and on chromatography paper. Oxidation reactions of o-dianisidine and o-phenylenediamine by H_2O_2 were the indicators (Shekhovtsova et al. 1997). One of the approaches was based on ammonia gas sensitive optode and an ammonium ion sensitive optode containing nonactin, an NH_4^+ selective ionophore. The efficiency of inhibition was most profound in the case of Ag(I) which had a detection limit of 0.02 ppm (0.18 lM), followed by Hg(II) and Cu(II) having detection limits of 0.07 ppm (0.35 lM) and 0.25 ppm (3.94 lM), respectively. Other metals were also investigated, but had higher limits of detection. The study also showed that the three metals mentioned had synergistic effects on inhibition, showing higher inhibition when present in combination (Preininger & Wolfbeis 1996). Later on mercury, copper, cadmium and zinc inhibition of urease was also examined using a coupled urease–glutamic dehydrogenase system as the biocomponent, with amperometric transduction. The lowest detectable

limits of Hg(II), Cu(II), Cd(II) and Zn(II) by the biosensor are 7.2 ppm (0.035 mM), 8.5 ppm (0.13 mM), 0.3 ppm (2.67 μM) and 0.2 ppm (3.05 μM), respectively, with a response time of 15 min (Rodriguez et al., 2004). Enzyme immobilization appears as a key factor to develop efficient biosensors with appropriate performances such as good operational and storage stability, high sensitivity, high selectivity, short response time, high reproducibility, economic convenience, higher stability, and the possibility to be easily removed from the reaction mixture leading to pure product isolation (Homaei et al., 2013).

Conclusion

The purpose of this review is to summarize the advances and trends in electrochemical biosensors for environmental applications. Biosensors are simple to use, cost-effective and portable useful analytical tools for environmental monitoring. Some examples of biosensors in advanced stage of development, which have been applied in cost-effective commercial devices, are given. Biosensors designed for measurement of specific chemicals are discussed. Heavy metal ions constitute a serious environmental problem due to their persistent and non-biodegradable nature which are toxic to biological systems even at low concentration. There is an obvious need to determine them at trace level. Conventional techniques to analyse metals include cold vapour atomic absorption spectrometry, inductively coupled plasma mass spectrometry, UV visible spectrophotometry and X-ray absorption spectroscopy. Thus, need arises for the fast and inexpensive methods for the detection of bioavailable heavy metals. Biosensors are useful analytical devices in this respect.

References

J. M. Mates, J. A. Segura, F. J. Alonso and J. Marquez, (2010). Roles of dioxins and heavy metals in cancer and neurological diseases using ROS-mediated mechanisms. *J. Free Radical Biol. Med.,* 49(9): 1328–1341.

K. Jomova and M. Valko (2011). Advances in metal-induced oxidative stress and human disease. *Toxicology*, 283:65–87.

Neelam Verma and Minni Singh (2005). Biosensors for heavy metals. *Biometals*, 18(2):121–129.

Abdou KA, Khadiga IA (2016). Biosensors for Monitoring of the Environmental Heavy Metals, 3(3): 31-38

Tekaya N, Saiapina O, Ouada H B, Lagarde F, Ouada H B and Jaffrezic-Renault N (2013). Ultra-sensitive conductometric detection of heavy metals based on inhibition of alkaline phosphatase activity from Arthrospira platensis. *Bioelectrochem*., 90: 24-29.

Chouteau C, Dzyadevych S, Chovelon J M and Durrieu C (2004). Development of novel conductometric biosensors based on immobilized whole cell *Chlorella vulgaris* microalgae. *Biosensors and Bioelectronics* 19: 1089-1096.

Chouteau C, Dzyadevych S, Durrieu C and Chovelon J M (2005). A bi-enzymatic whole cell conductometric biosensor for heavy metal ions and pesticides detection in water samples Biosens. Bioelectron., *Biosensors and Bioelectronics* 21: 273-281.

Gronow M (1984). Biosensors. *Trends in Biochemical sciences*, 9(8): 336-340.

D'Souza SF (2001). Microbial biosensors. *Biosens Bioelectron,* **16:** 337-353.

Verma N and Singh M (2003). A disposable microbial based biosensor for quality control in milk. *Biosens Bioelectron*, 18:1219-1224.

Verma N and Malaku ET (2001). Studies on the development of disposable biosensor for monitoring malathion pesticide residues. In: Biochemistry-Environment and Agriculture. Ed. APS Mann; 265-269.

Prasad S, Zhang X, Yang M, Ozkan CS and Ozkan M (2004). Neurons as sensors: Individual and cascade chemical sensing. *Biosens Bioelectron*, 19: 1599-1610.

Zhang L, Liu L and Xia N (2013). Electrochemical Sensing of Alkaline Phosphatase Activity Based on Difference of Surface Charge of Electrode. *Int. J. Electrochem. Sci.,* 8: 8311-8319.

Turdean G (2011). Design and Development of Biosensors for the Detection of Heavy Metal Toxicity. *International Journal of Electrochemistry*, 2011:1-15. doi:10.4061/2011/343125.

Homaei A (2015). Purification and biochemical properties of highly efficient alkaline phosphatase from Fenneropenaeus merguiensis brain. *Journal of Molecular Catalysis B Enzymatic*, 118:16-22.

Homaei A (2017). Immobilization of Penaeus merguiensis alkaline phosphatase on gold nanorods for heavy metal detection. *Ecotoxicology and Environmental Safety*, 136:1-7.

Van Dyk J S and Pletschke B (2011). Review on the use of enzymes for the detection of organochlorine, organophosphate and carbamate pesticides in the environment. *Chemosphere*, 82(3):291-307.

Guascito M R, Malitesta C, Mazzotta E and Turco A (2008). Inhibitive determination of metal ions by an amperometric glucose oxidase biosensor: study of the effect of hydrogen peroxide decomposition. *Sensors and actuators B,* 131:394-402.

Zhylyak G A, Dzyadevich S V, Korpan Y I, Soldatkin A P and. El'skaya (1995). Application of urease conductometric biosensor for heavy-metal ion determination. *Sensors and Actuators* B, 24-25: 145-148.

Li J, Tan S N and Oh J T (1998). Silica sol-gel immobilized amperometric enzyme electrode for peroxide determination in the organic phase. *Journal of Electroanalytical Chemistry*, 448: 69-77.

Ho W O, Krause S, McNeil C J, Pritchard J A, Armstrong R D, Athey D and Rawson K (1999). Electrochemical sensor for measurement of urea and creatinine in serum based on AC impedance measurement of enzyme-catalysed polymer transformation. *Anal. Chem.*, 71(10): 1940-1946.

Srivastava P K, Kayastha A M and Srinivasan (2001). Characterization of gelatin-immobilized pigeonpea urease and preparation of a new urea biosensor. *Biotechnol Appl Biochem*., 34(1):55-62.

Ilangovan R, Daniel D, Krastanov A, Zachariah C and ElizabethR (2006). Enzyme Based Biosensor For Heavy Metal Ions Determination. *Biotechnol. & Biotechnol,* 20:184-189.

Satoh I (1991). An apoenzyme thermistor microanlaysis for zinc (II) ions with use of an immobilized alkaline phosphatase reactor in a flow system. *Biosens Bioelectron,* 6(4): 375-379.

Gayet J C, Haouz A, Meyer A and Burstein C (1993). Detection of heavy metal salts with biosensors built with an oxygen electrode coupled to various immobilized oxidases and dehydrogenases. *Biosens Bioelectron*, 8: 177-183.

Cowell D C, Dowman A A and Ashcroft T (1995). The detection and identification of metal and organic pollutants in potable water using enzyme assays suitable for sensor development. *Biosens Bioelectron* ,10(6-7): 509–516.

Shekhovtsova T N, Muginova S V and Bagirova N A (1997). Determination of organomercury compounds using immobilized peroxidase. *Anal Chim Acta*, 344: 145-151.

Preininger C and Wolfbeis O (1996). Disposable cuvette test with integrated sensor layer for enzymatic determination of heavy metals. *Biosens Bioelectron*, 11: 981-990.

Rodriguez B B, Borbot J A and Tothill I E (2004). Development of urease and glutamic dehydrogenase amperometric assay for heavy metals screening in polluted samples. *Biosens Bioelectron*, 19(1): 1157-1167.

Homaei A A, Sariri R, Vianello F and Stevanato R (2013). Enzyme immobilization: an update. *J Chem Biol*, 6(4): 185-205.