

# Biosensors for Environmental Applications

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## 1. Introduction

The increasing number of potentially harmful pollutants in the environment calls for fast and cost-effective analytical techniques to be used in extensive monitoring programs. Additionally, over the last few years, a growing number of initiatives and legislative actions for environmental pollution control have been adopted in parallel with increasing scientific and social concern in this area [1-4]. The requirements for application of most traditional analytical methods to environmental pollutants analysis, often constitute an important impediment for their application on a regular basis. The need for disposable systems or tools for environmental applications, in particular for environmental monitoring, has encouraged the development of new technologies and more suitable methodologies. In this context, biosensors appear as a suitable alternative or as a complementary analytical tool. Biosensors can be considered as a subgroup of chemical sensors in which a biological mechanism is used for analyte detection [1,3-4].

A biosensor is defined by the International Union of Pure and Applied Chemistry (IUPAC) as a self-contained integrated device that is capable of providing specific quantitative or semi-quantitative analytical information using a biological recognition element (biochemical receptor), which is retained in contact direct spatial with a transduction element [5]. Biosensors should be distinguished from bioassays where the transducer is not an integral part of the analytical system [4-7]. Biosensing systems and methods are being developed as suitable tools for different applications, including bioprocess control, food quality control, agriculture, military and in particular, for medical applications.

Biosensors are usually classified according to the bioreceptor element involved in the biological recognition process (e.g., enzymes, immunoaffinity recognition elements, whole-cells of micro-organisms, plants or animals, or DNA fragments), or according to the physicochemical transducer used (e.g., electrochemical, optical, piezoelectrical or thermal). The main classes of bioreceptor elements that are applied in environmental analysis are whole cells of microorganisms, enzymes, antibodies and DNA. Additionally, in the most of the biosensors described in the literature for environmental applications electrochemical transducers are used [5].

For environmental applications, the main advantages offered by biosensors over conventional analytical techniques are the possibility of portability, miniaturization, work on-site, and the ability to measure pollutants in complex matrices with minimal sample

preparation. Although many of the developed systems cannot compete yet with conventional analytical methods in terms of accuracy and reproducibility, they can be used by regulatory authorities and by industry to provide enough information for routine testing and screening of samples [1,3,8].

Biosensors can be used as environmental quality monitoring tools in the assessment of biological/ecological quality or for the chemical monitoring of both inorganic and organic priority pollutants. In this review article we provide an overview of biosensor systems for environmental applications, and in the following sections we describe the various biosensors that have been developed for environmental monitoring, considering the pollutants and analysis that are usually mentioned in the literature.

## 2. Heavy metals

Heavy metals are currently the cause of some of the most serious pollution problems. Even in small concentrations, they are a threat to the environment and human health because they are non-biodegradable. People are constantly been exposed to heavy metals in the environment [8-9]. The dangers associated with heavy metals are due to the ubiquitous presence of these elements in the biosphere, their bioavailability from both natural and anthropogenic sources, and their high toxicity. Thus, there are several cases described in the literature where exposure of populations to these pollutants has resulted in severe damage to their health, including a significant amount of deaths. The metal contaminants most commonly observed in the environment are: lead, chromium, zinc, mercury, cadmium and copper [10-11].

Conventional analytical techniques for heavy metals (such as cold vapour atomic absorption spectrometry, and inductively coupled plasma mass spectrometry) are precise, but suffer from the disadvantages of high cost, the need for trained personnel and the fact that they need to be performed in the laboratory [9]. For the reasons cited earlier, biosensors are being developed and utilized for monitoring heavy metal concentrations in environmental samples [9,12-14]. Furthermore, their biological basis makes them ideal for toxicological measurement of heavy metals, while conventional techniques can only measure concentrations [15].

Many of the bacterial biosensors developed for analysis of heavy metals in environmental samples, make use of specific genes responsible for bacterial resistance to these elements, such as biological receptors. Bacterial strains resistant to a number of metals such as zinc, copper, tin, silver, mercury and cobalt have been isolated as possible biological receptors [16-18]. The metal resistance of these genes is induced only when the element reaches the bacteria's cytoplasm. The specificity of this resistance mechanism contributes to the construction of cell biosensors for detection of metals from the fusion of these resistance genes with genes encoding bioluminescent proteins, for example, luciferin. In this case, the production of light, which can be measured by luminometers and photometers, indicates the presence of a heavy metal in the sample [9,17].

Enzymatic methods are also commonly used for metal ion determination, since these can be based on the use of a wide range of enzymes that are specifically inhibited by low concentrations of certain metal ions [19-20]. Domínguez-Renedo et al. [21] developed enzymatic amperometric biosensors for the measurement of Hg<sup>2+</sup>, based on the inhibitory action of this ion on urease activity. They used screen-printed carbon electrodes as support and screen-printed carbon electrodes modified with gold nanoparticles. The same enzyme

was used by Kuswandi [22] in the development of a simple optical fibre biosensor for the determination of various heavy metal ions: Hg(II), Ag(I), Cu(II), Ni(II), Zn(II), Co(II) and Pb(II). Durrieu and Tran-Minh [23] developed an optical biosensor to detect lead and cadmium by inhibition of alkaline phosphatase present on the external membrane of *Chlorella vulgaris* microalgae, used as biological recognition element. Moreover, a biosensor with microalgae *Tetraselmis chui* was developed for the voltammetric measurement of Cu<sup>2+</sup> for Alpat et al. [24]. The developed algae-based biosensor was also successfully applied to the determination of copper (II) in real sample and the results were confirmed when compared to those obtained by atomic absorption spectrophotometric method. Table 1 lists some examples of biosensors developed for heavy metal determination.

Analyte	Recognition biocomponent	Transduction system	Matrix	Ref.
Zinc, copper, cadmium and nickel	<i>Pseudomonas fluorescens</i> 10586s pUCD607 with the <i>lux</i> insertion on a plasmid	Optical (luminometer)	Soil	[25]
Cadmium	DNA	Electrochemical	Standard solutions	[26]
Cadmium	Phytochelatin	Optical (localized surface plasmon resonance)	Standard solutions	[27]
Mercury, cadmium and arsenic	Urease enzyme	Electrochemical	Standard solutions	[28]
Nickel ions	<i>Bacillus sphaericus</i> strain MTCC 5100	Electrochemical	Industrial effluents and foods	[29]
Zinc, copper, cadmium, nickel, lead, iron and aluminum	<i>Chlorella vulgaris</i> strain CCAP 211/12	Electrochemical	Urban waters	[30]
Cadmium	<i>Escherichia coli</i> RBE23-17	Electrochemical	Wastewater	[31]
Zinc, cobalt and copper	<i>Pseudomonas</i> sp. B4251, <i>Bacillus cereus</i> B4368 and <i>E. coli</i> 1257	Electrochemical	Water	[32]
Mercury (II) and lead (II) ions	DNA	Optical	Water	[33]
Copper (I) and (II) ions	DsRed (red fluorescent) protein	Optical	Standard solutions	[34]
Cadmium, copper and lead	Sol-gel-immobilized-urease	Electrochemical	Synthetic effluents	[35]

Table 1. Examples of biosensors developed for heavy metal determination.

### 3. Biochemistry Oxygen Demand (BOD)

Biochemical oxygen demand (BOD or BOD<sub>5</sub>) is a parameter widely used to indicate the amount of biodegradable organic material in water. Its determination is time consuming,

and consequently it is not suitable for online process monitoring. Fast determination of BOD could be achieved with biosensor-based methods. Most BOD sensors rely on the measurement of the bacterial respiration rate in close proximity to a transducer, commonly of the Clark type (an amperometric sensor developed by Clark in 1956 for measuring dissolved oxygen) [2].

BOD biosensors are the most common commercial biosensors for environmental monitoring. The first commercial BOD sensor was produced by the Japanese company Nisshin Electric in 1983 and a number of other commercial BOD biosensors based on microbial cells are being marketed by Autoteam GmbH, Medingen GmbH and Dr. Lange GmbH in Germany; Kelma (Belgium); Bioscience, Inc. and US Filter (USA) [2,4,12].

Nakamura and Karube [36] developed a system for measuring BOD from cells of recombinant *Escherichia coli* with *Vibrio fischeri* genes lux AE. With this system the real time analysis of multiple samples was possible. These handy devices have been marketed primarily for food and pharmaceutical industries. Moreover, an optical biosensor for parallel multi-sample determination of biochemical oxygen demand in wastewater samples has been developed by Kwok *et al.* [37]. The biosensor monitors the dissolved oxygen concentration in artificial wastewater through an oxygen sensing film immobilized on the bottom of glass sample vials. Then, the microbial samples were immobilized on this film and the BOD value was determined from the rate of oxygen consumption by the microorganisms in the first 20 minutes.

#### 4. Nitrogen compounds

Nitrites are widely used for food preservation and for fertilization of soils. However, continuous consumption of these ions can cause serious implications on human health, particularly because it can react irreversibly with hemoglobin [38]. The increasing levels of nitrate found in groundwater and surface water are of concern because they can harm the aquatic environment. In line with this, the regulations for treatment of urban wastewater in order to reduce pollution, including pollution by nitrates, from sewage treatment works of industrial and domestic, have been implemented [2].

Chen *et al.* [39] developed a biosensor for amperometric determination of nitrite using cytochrome c nitrite reductase (ccNiR) from *Desulfovibrio desulfuricans* immobilized and electrically connected on a glassy carbon electrode by entrapment into redox active [ZnCr-AQS] double layered hydroxide containing anthraquinone-2-sulfonate (AQS). The instrument showed a fast response to nitrite (5 seconds) with a linear range between concentrations of nitrite 0.015 and 2.35  $\mu\text{M}$  and a detection limit of 4nM.

A highly sensitive, fast and stable conductimetric enzymatic biosensor for the determination of nitrate in waters was described in Wang *et al.* [40-41]. Conductimetric electrodes were modified by methyl viologen mediator mixed with nitrate reductase from *Aspergillus niger* by cross-linking with glutaraldehyde in the presence of bovine serum albumin and Nafion® cation-exchange polymer, allowing retention of viologen mediator. A linear calibration curve in the range of 0.02 and 0.25 mM with detection limits of 0.005 mM nitrate was obtained. When stored in pH 7.5 phosphate buffer, the sensors showed good stability over two weeks. Moreover, Khadro *et al.* [42], developed an enzymatic conductimetric biosensor for the determination of nitrate in water, validated and used for natural water samples. The instrument was based on a methyl viologen mediator mixed with nitrate reductase from *Aspergillus niger* and Nafion® cation-exchange polymer dissolved in a plasticized PVC

membrane deposited on the sensitive surface of interdigitated electrodes. When stored in phosphate buffer pH 7.5 at 4°C, the sensor showed good stability over 2 months.

## 5. PCBs

Polychlorinated biphenyls (PCBs) are toxic organic compounds [43-44] that are ubiquitous environmental pollutants, even though their production was banned in several countries many years ago [44]. It is currently assumed that food is the major source of the PCB exposure since PCBs are highly lipophilic and accumulate in the food chain, so foods of animal origin are an important source of exposure. The level of PCBs in the environment depends on the matrix where it originated [43,45].

There are 209 polychlorinated biphenyl congeners that persist worldwide in the environment and food chain. These congeners are divided into three classes based upon the orientation of the chlorine moieties, *i.e.*, coplanar, mono-*ortho* coplanar, and non-coplanar [43]. Conventional techniques used for the analysis of PCBs are generally based on gas chromatography coupled with mass spectrometry (GC/MS) [43,45]. Alternative techniques based, for example, on immunoassays, are inexpensive and rapid screening tools for sample monitoring in laboratory and field analysis. Moreover, immunoassays are simple, sensitive, reliable, and relatively selective for PCBs testing. Among several immunoassay techniques, the enzyme-linked immunosorbent assay (ELISA) combined with colorimetric end-point detection are the most popular. Another interesting approach is the use of immunosensor technology [45]. Immunosensors are a class of biosensors that use as biological recognition elements, antibodies or antigens [45]. Pribyl *et al.* [44] developed a novel piezoelectric immunosensor for determination of PCB congeners in the range of concentrations usually found in real matrices (soil). The presented method allows one to carry out analysis of extracts directly without any additional purification steps. Moreover Gavlasova *et al.* [45] had a successful application of a sol-gel silica entrapment of viable *Pseudomonas sp.* P2 cells for constructing low-cost sensors for environmental monitoring using real soil.

## 6. Phenolic compounds

A considerable number of organic pollutants, which are found widely distributed in the environment, have phenolic structures. Phenols and their derivatives, are well known because of their high toxicity and are common compounds in industrial effluents, coming from the activities related to production of plastics, dyes, drugs, antioxidants, polymers, synthetic resins, pesticides, detergents, desinfectants, oil refinery and mainly pulp and paper [46].

Several substituted phenols, such as chloro- and nitrophenols, are highly toxic to humans and aquatic organisms [47-48]. These two groups of substituted phenols are the main degradation products of organophosphorus pesticides and chlorinated phenoxyacids. Even at small concentrations (< 1 ppm), phenolic compounds affect the taste and odor of drinking water and fish [47]. Many of these compounds have toxic effects in animals and plants, because they easily penetrate the skin and cell membranes, determining a wide range of genotoxicity, mutagenicity, hepatotoxic effects, and affect the rate of biocatalyzed reactions, and the processes of respiration and photosynthesis [2]. Thus, phenols and specially their chlorinated, nitro and alkyl derivatives have been defined as hazardous pollutants due to their high toxicity and persistence in the environment, and are found in the list of hazardous

substances and priority pollutants of the EC (European Commission) and the U.S. Environmental Protection Agency (EPA).

The toxic pollutants in wastewater usually interact with DNA, leading to the damage to human health, but these very interactions between these toxic pollutants and DNA can be used in electrochemical DNA biosensors, generating a response signal, thus providing an effective approach for rapid screening of pollutants. Based on this principle, various electrochemical DNA sensors for environmental monitoring have been proposed. It has been demonstrated that DNA-based devices hold great promise for environmental screening of toxic aromatic compounds and for elucidating molecular interactions between intercalating pollutants and DNA. Using a disposable electrochemical DNA biosensor made by immobilizing double stranded DNA onto the surface of a disposable carbon screen-printed electrode, toxicants in water and wastewater samples have been successfully detected, which correlates well with the classic genotoxicity tests based on bioluminescent bacteria [49-53]. Parellada *et al.* [54] developed an amperometric biosensor with tyrosinase (a polyphenol oxidase with a relatively wide selectivity for phenolic compounds) immobilized in a hydrogel on a graphite electrode, which correlated satisfactorily with the official method for the determination of the phenol index in environmental samples. Chlorophenols have been also detected with a chemiluminescence fibre-optic biosensor adapted a flow injection analysis (FIA).

Phenolic compounds widely distributed in the environment as organic pollutants can be oxidized by conventional carbonaceous electrodes generally in high voltage (0.8 V *vs.* ECS). Under such over-voltage conditions, these compounds can dimerize and produce other electroactive species (radicals), resulting in higher than expected electrical current levels. In other cases, there may be adsorption or formation of polymeric products with consequent passivation of the electrode, leading to the observation of peaks with intensities very below those expected. In these cases the high applied potential may increase background current levels, and consequently the level of noise. Thus, by the use of electrodes modified with oxidase enzymes, coupled with the principle of biochemical oxidation followed by electrochemical reduction, one can undo or minimize these variables. Enzymes commonly used in the manufacture of biosensors are the laccase, tyrosinase and peroxidases [55-56].

Analyte	Recognition enzyme	Transduction system	Matrix	Ref.
Binary mixtures: phenol/clorophenol, catechol/phenol, cresol/clorocresol and phenol/cresol	Laccase and tyrosinase	Amperometric multicanal	wastewater	[37]
m-cresol or catechol	DNA	Amperometric	wastewater	[30]
Phenol	Mushroom tissue (tyrosinase)	Amperometric	wastewater	[38]
phenol, p-cresol, m-cresol and catechol	Polyphenol oxidase	Amperometric	wastewater	[39]

Table 2. Some of the most commonly used biosensors in phenolic compounds

Regarding the selectivity of biosensors based on peroxidase, greater sensitivity to the compounds 2-amino-4-chlorophenol and 4-chloro-3-methylphenol was observed among 20 compounds tested [57]. While the action of tyrosinase is confined largely to phenol and *ortho*-benzenediols the laccases are able to oxidize various phenolic substrates, including phenols and diphenols (*ortho*-, *meta*- and *para*-benzenediols), phenols, catecholamines, *etc.*, Liu *et al.* [58] developed a biosensor for phenol based on the immobilization of tyrosinase on the surface of modified magnetic MgFe<sub>2</sub>O<sub>4</sub> nanoparticles. Table 2 presents some examples of biosensors used in the detection of phenolic compounds in wastewater matrices.

## 7. Endocrine disruptors and hormones

Endocrine disruptors, exogenous compounds that alter the endogenous hormone homeostasis, have been systematically discharged in the environment during the last years [62]. These contaminants have been related to the decrease of human sperm numbers and increased incidence of testicular, breast and thyroid cancers. These endocrine disruptors can act by the following mechanisms: a) inhibition of enzymes related to hormone synthesis; b) alteration of free concentration of hormones by interaction with plasmatic globulins; c) alteration in expression of hormone metabolism enzymes; d) interaction with hormone receptors, acting as agonists or antagonists; e) alteration of signal transduction resulting from hormone action. The importance of the identification of endocrine disruptors involves characterization of environmental contaminants and inquiry of new substances discharged in the environment.

Natural and synthetic hormone residues can be found in the environment as a result of human or animal excretion due to population growing and more intensive farming. Hormones such as estradiol, estrone and ethynylestradiol have been found in water at ng/L levels, but even at these low concentrations, some of them may have endocrine-disrupting activity in aquatic or even terrestrial organisms. Estrone, progesterone and testosterone, along with other organic pollutants, have been determined with a fully automated optical immunosensor in water samples, reaching limits of detection up to sub-ng/L [63].

An electrochemical biosensor for progesterone in cow's milk was developed and used in a competitive immunoassay by Xu *et al.* [64]. The sensor was fabricated by depositing anti-progesterone monoclonal antibody (mAb) onto screen-printed carbon electrodes (SPCEs) which were coated with rabbit anti-sheep IgG (rIgG). This sensor was operated following the steps of competitive binding between sample and conjugate (alkaline-phosphatase-labelled progesterone) for the immobilised mAb sites and measurements of an amperometric signal in the presence of *p*-nitrophenyl phosphate using either colorimetric assays or cyclic voltammetry [64].

## 8. Organophosphorus compounds (OP)

Organophosphorus (OP) compounds are a group of chemicals that are widely used as insecticides in modern agriculture for controlling a wide variety of insect pests, weeds, and disease-transmitting vectors [65].

### 8.1 Pesticides

A pesticide, as defined by the EPA, is any substance or mixture of substances intended for preventing, destroying, repelling, or lessening the damage of any pest, [65]. Of all the

environmental pollutants, pesticides are the most abundant, present in water, atmosphere, soil, plants, and food [2].

Concerns about the toxicity, ubiquity and persistence of pesticides in the environment have led the European Community to set limits on the concentration of pesticides in different environmental waters. Directive 98/83/EC on the quality of water for human consumption has set a limit of 0.1 µg/L for individual pesticides and of 0.5 µg/L for total pesticides. Enzymatic sensors, based on the inhibition of a selected enzyme are the most extensively used biosensors for the determination of these compounds [66].

Parathion (*O,O*-diethyl-*O*-4-nitrophenyl thiophosphate), is a broad-spectrum OP pesticide having a wide range of applications against numerous insect species on several crops. Parathion is also used as a preharvest soil fumigant and foliage treatment for a wide variety of plants, both in the field and in the greenhouse. Parathion is highly toxic by all routes of exposure – ingestion, skin adsorption, and inhalation – all of which have resulted in human fatalities. Like all pesticides, parathion irreversibly inhibits AChE [67]. Table 3 presents a few examples of biosensors for determination of pesticides, including parathion.

## 8.2 Herbicides

For the detection of herbicides such as the phenylureas and triazines, which inhibit photosynthesis, biosensors have been designed with membrane receptors of thylakoid and chloroplasts, photosystems and reaction centers or complete cells such as unicellular alga and phenylureas and triazines, in which mainly amperometric and optical transducers have been employed [68]. Table 3 also presents some examples of biosensors used in the detection of herbicides.

Analyte	Type of interaction	Recognition biocatalyzer	Transduction system	Ref.
<b>Pesticides</b>				
Simazina	Biocatalytic	Peroxidase	Potentiometric	[47]
Isoproturon	Biocatalytic	Antibody encapsulate	Immunosensor immunoreaction	[48]
Parathion	Biocatalytic	Parathion hydrolase	Amperometric	[50]
Paraoxon	Biocatalytic	Alkaline phosphatase	Optical	[46,50]
Carbaril	Biocatalytic	Acetilcolinesterase	Amperometric	[51]
<b>Herbicides</b>				
2,4-Diclorofenoxiacetic	Immunoanalysis	Acetilcolinesterase	Amperometric	[52]
Diuron, Paraquat,	Biocatalytic	Cyanobacterial	Bioluminescence	[53]

Table 3. Biosensors used in the detection of pesticides and herbicides.

## 8.3 Dioxins

Dioxins are potentially toxic substances for humans with a major impact on the environment that can reach the food chain accidentally, as contaminating residues present in water and

soil. Dioxins are organosoluble, toxic, teratogenic and carcinogenic. They are unintended by-products of many industrial processes where chlorine and chemicals derived from it are produced, used and disposed of. Industrial emissions of dioxin to the environment can be transported over long distances by airstreams and, less importantly, by rivers and sea currents. Consequently, dioxins are now widely present all over the globe. It is estimated that even if production completely stopped today, the current environmental levels would take years to diminish. This is because dioxins are persistent, taking years to centuries to deteriorate, and can be continuously recycled in the environment [69]. Biosensors for detection and monitoring of these pollutants in the area would be extremely useful.

## 9. Commercial activities

About 200 companies worldwide were working in the area of biosensors and bioelectronics at the turn of the century. Some of these companies are still involved in biosensor fabrication/marketing, whereas others just provide the pertinent materials and instruments for biosensor manufacture. Most of these companies are working on existing biosensor technologies and only a few of them are developing new technologies [76]. The application of new biodevices to real-world environmental samples is a must in the final steps of development. However, despite of the great number of newly developed biosensors, most literature references overlook the real-world step and only report applications of the biosensor in either distilled water or buffered solutions.

Most of the reviewed systems still have some way to go before application to real samples can be made, and the study of matrix effects, stability issues and careful comparison with established methods are crucial steps in this approach. Even though commercial returns from environmental biosensors are substantially less than from medical diagnostics, public concern and government funding have generated a major research effort aimed at the application of biosensors to the measurement of pollutants and other environmental hazards [77].

## 10. Other applications

Many biosensors reported for environmental applications show the potential to be developed for either single-sample formats for field screening applications or continuous formats for field monitoring applications. A discussion concerning the cleanup of a Superfund site may provide examples of the scope and kinds of environmental screening and monitoring problems for which biosensors could provide unique solutions

Most of the work on metal-specific bacterial sensors has been done by using a liquid suspension of viable sensor bacteria. However, a more advanced approach is to use these bacteria in the biosensor system, e.g., by immobilising the cells onto optical fibres connected to a photo detection device. This type of fibre-optic biosensors have been previously constructed for Cu [76], genotoxicants, or general toxicity of industrial effluents [78]. These fibre-optic sensors can easily be brought to the field and used for on-line monitoring.

Ivask *et al* (2006) developed fibre-optic biosensors for the analysis of environmental samples, e.g., soils and sediments *in situ*. For that, the existing recombinant luminescent *Escherichia coli* MC1061 (pmerRluxCDABE) and MC1061 (parsluxCDABE) [79] responding specifically to Hg and As, respectively, were immobilised onto optical fibres in order to develop self contained biosensors. The system was optimised for the Hg biosensor and the derived

protocol was used for analysing bioavailable Hg and As in natural soil or sediment suspensions. The biosensors consist of alginate-immobilised recombinant bacteria emitting light specifically in the presence of bioavailable Hg or As in a dose-dependent manner. The biosensors alongside with the non-immobilised Hg and As sensor bacteria were successfully applied for the analysis of bioavailable fractions of Hg and As in soil and sediment samples from the Aznalcollar mining area (Spain).

Haron *et al* (2006) developed three layer waveguiding silicon dioxide (SiO<sub>2</sub>)/silicon nitride (Si<sub>3</sub>N<sub>4</sub>)/SiO<sub>2</sub> structure on silicon substrate was proposed as an optically efficient biosensor for calibration of heavy metal ions in drinking water. Total attenuated reflection (ATR) in portable and miniaturized SiO<sub>2</sub>/Si<sub>3</sub>N<sub>4</sub>/SiO<sub>2</sub> waveguides was successfully exploited in the present investigation for development of a highly sensitive biosensors and the detection limit as low as 1 ppb was achieved for Cd<sup>2+</sup> and Pb<sup>2+</sup> ions. Composite membranes containing both biologically active components, e.g. enzymes, and organic chromophores (indicators) were formed using the polyelectrolyte self-assembly deposition technique. The latter are sensitive to small changes in local pH caused by enzyme reactions, and thus provided a transducing function. The difference in inhibition of enzymes urease and acetylcholine esterase by the heavy metal ions established the possibility of designing a sensor array for discrimination between different types of water pollutants and the device is light, portable and robust

## 11. Conclusions

Most biosensor systems have been tested only on distilled water or buffered solutions, but more biosensors that can be applied to real samples have appeared in recent years. In this context, biosensors for potential environmental applications continue to show advances in areas such as genetic modification of enzymes and microorganisms, improvement of recognition element immobilization and sensor interfaces.

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