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Review

# Cholinesterase-based biosensor for preliminary detection of toxic heavy metals in the environment and agricultural-based products

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**Abstract**

Heavy metals with high chemical activity from sludge and waste release, agriculture, and mining activity are a major concern. They should be carefully managed before reaching the main water bodies. Excessive exposure to heavy metal may cause toxic effect to any types of organism from the biomolecular to the physiological level, and ultimately cause death. Monitoring is the best technique to ensure the safety of our environment before a rehabilitation is needed. Nowadays, enzyme-based biosensors are utilised in biomonitoring programmes as this technique allows for a real-time detection and rapid result. It is also inexpensive and easy to handle. Enzyme-based biosensors are an alternative for the preliminary screening of contamination before a secondary screening is performed using high-performance technology. This review highlights the current knowledge on enzyme-based biosensors, focusing on cholinesterase for toxic metal detection in the environment.



## Introduction

Several heavy metals work as a cofactor in metabolic functions in the living systems. Moreover, they can trigger a number of responses only in trace amounts. Examples of metabolic processes that require heavy metals as a mediator are the thermal stability regulation of human ceruloplasmin (Sedlák *et al.,* 2008), redox reaction (Miranda *et al.,* 2000), mitochondrial respiration, iron absorption, free radical scavenging, and elastin crosslinking (Salvamani *et al.,* 2016). Unfortunately, low and high concentration of heavy metals, either in the form of ion or salt in a biological system, will disrupt and slow down some reactions, thus damaging the main function of the system (Sabullah *et al.,* 2015). Heavy metal excess has become a public concern as compared to heavy metal deficiencies because of the adverse effect towards individuals and also possibility to be released into the environment (Ralph and McArdle, 2001). The major sources of heavy metal pollution are heavy industrial activities, agriculture, and mining. However, other sources of toxic metal ions have also been recorded from various types of industries, which are summarised in Table 1. Heavy metals exist in the ionic state which accidentally leads to leaching or being carried by runoff water into nearby waterways, eventually flowing into water resources. Aquatic organisms have high chances to be exposed to these contaminants, and the ones that do will exhibit a variety of symptoms, particularly a decrease in swimming activity (Waser *et al.,* 2009), critical avoidance behaviour (Lopes *et al.,* 2004; Padrilah *et al.,* 2017), and reduced appetite, all of which affect their growth performance (Ali *et al.,* 2003), which may then induce death (Lauer *et al.,* 2012). This situation depends on the concentration levels and time of exposure (Dornfeld *et al.,* 2009).

Scopus indexed the number of papers published during 2008 - 2017 based on heavy metal toxicity, which has been increasing by year (Figure 1). Studies on lead (Pb) toxicity have the highest number of publications with a total of 22,884 papers in 10 years, followed by zinc (Zn), copper (Cu), cadmium (Cd), arsenic (As), mercury (Hg), chromium (Cr), and nickel (Ni) at 9,333, 9,133, 8,099, 5,156, 3,647, 3,567, and 3,269, respectively. Based on fold popularity from 2008 to 2017, studies on Pb, Cd, Cr, Ni, and Zn toxicity increased more than two-fold as compared to those on Cu, Hg, and As. However, a review by Tierney *et al.* (2010) focused more on copper toxicity as this compound gives a negative impact on biological systems at a concentration lower than 10 µg/L. Tchounwou *et al.* (2012) mentioned that Hg ranks the highest in heavy metal toxicity, and other studies have proved this

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Table 1. Source of metal-based pollutants from various industries.

| Industry | Metal | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | As | Cd | Cr | Cu | Hg | Ni | Pb | Zn |
| Mining activity and ore processing | √ | √ | | | √ | | √ | |
| Alloys, electroplating, and metallurgy | √ | √ | √ | √ | √ | √ | √ | √ |
| Chemical industries | √ | √ | √ | √ | √ | | √ | √ |
| Ink, dye, and pigment manufacturing | √ | √ | | √ | √ | √ | √ | |
| Glass, ceramic, and porcelain | √ | | √ | | | | | |
| Print and photography | | √ | √ | | | √ | √ | √ |
| Pulp and paper mills | | | √ | √ | √ | | | |
| Leather tanning | √ | | √ | √ | √ | | | √ |
| Drugs and pharmaceuticals | | | | √ | √ | | | |
| Fabric and textile industry | √ | √ | | √ | √ | √ | | |
| Nuclear technology | | √ | | | | | | |
| Pesticides and fertilisers | √ | √ | √ | √ | √ | √ | | √ |
| Chloralkali process | √ | √ | √ | | √ | | | √ |
| Oil refining | √ | √ | √ | | √ | √ | | √ |

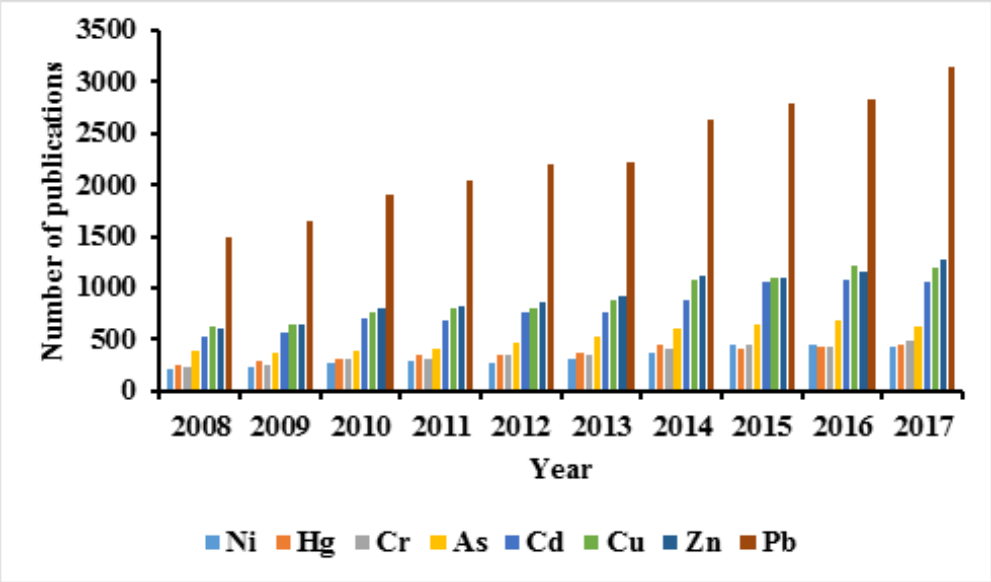


Figure 1. The number of papers published during 2008 - 2017. The search was carried out on Scopus by using eight research queries: nickel, mercury, chromium, arsenic, cadmium, copper, zinc, and lead toxicity (Scopus, August 2018).

statement, such as those by Apartin and Ronco (2001), Bellas *et al.* (2001), and Ramakritinan *et al.* (2012).

However, other reports prove that Cu is the most toxic metal, such as the study done by Nałecz-Jawecki and Sawicki (1998), which showed that Cu is the most toxic metal on protozoans, followed by silver (Ag) and Hg, based on lethal response evoked by these compounds. The toxic effects of copper are proven by *Daphnia magna* (planktonic crustacean), which was sensitive towards this compound at a lower $LC_{50}$ value as compared to Zn and lead Pb (Arambasic and Bjelic, 1995). Hutchinson and Williams (1994) also proved that a lower Cu concentration caused 50% mortality in *Cyprinodon variegatus* (sheepshead pupfish) as compared to Cd and chromium Cr. Regarding the effects of heavy metals on plants, Fargašová (2004) reported that Cu showed the highest inhibition on *Sinapis alba* (white mustard) growth, followed by Cd, iron (Fe), Zn, and Pb. Manusadžianas *et al.* (2002) suggested that Hg, Cu, and Cd are in the group of the most toxic metal, as shown by their adverse effect on Nitellopsis obtusa (alga) at the cellular, cell membrane, and enzyme levels. On the other hand, a study by Bat *et al.* (1999), based on the calculation of lethality time ($LT_{50}$) on *Idotea balthica* (marine isopod), reported that Zn was the most toxic compound as compared to Cu and Pb, and Pb was less toxic on this species. Toxicology bioassay on the mortality rate ($EC_{50}$) of two

marine microalgae, *Isochrysis galbana* and *Tetraselmis chui*, proved that Cu had toxic effect on these species at a lower concentration as compared to Pb (Liu *et al.,* 2011). Verslycke *et al.* (2003) also reported that Pb was less toxic as compared to Cu based on acute 96 h toxicity tests on *Neomysis integer* (opossum shrimp). Arsenic (As) toxicity is also of concern; the trivalent form of this metal ion generally has higher solubility in water and is more toxic as compared to pentavalent As (Hughes *et al.,* 2011). Similarly, $As^{3+}$ exposure on human cells resulted in higher cytotoxicity as compared to $As^{5+}$ (Styblo *et al.,* 2000). Liebl *et al.* (1995) proved the capability of $As^{3+}$ to block gluconeogenesis and inhibit glucose uptake in rat kidney tubules, while $As^{5+}$ had no significant effect. Thus, more studies need to be conducted to reduce the hazardous impact to living organisms and their habitat.

*Biosensor development*

Inductively coupled plasma atomic emission spectroscopy (ICP-AES), inductively coupled plasma mass spectrometry (ICP-MS), and inductively coupled plasma optical emission spectrometry (ICP-OES) are the current mainstream test methods to identify and quantify the presence of metal ion in any samples. Shi *et al.* (2009) used ICP-AES to quantify trace elements in wheat grain, while Rui *et al.* (2008a) used ICP-MS to distinguish heavy metal contents in two different nitrogen fertilisers, ammonium sulphate-based and urea-based fertilisers. Other than that, ICP was used to determine heavy metal contamination level in soil and crop along the Yellow River basin (Rui *et al.,* 2008b). Unfortunately, this sensitive analytical method is too expensive, consumes much time, and needs skilful technician to operate the whole system. Nowadays, biomarkers have been developed as an alternative to measure the effect or stress level of heavy metals on the environmental. This test is a preliminary screening, which consists of various test levels from the molecular origin to physiological observation. For example, fish have been utilised as a biomarker to determine water contamination based on their biochemical reaction, cellular alteration, and behaviour (Albertsson *et al.,* 2007; Farombi *et al.,* 2007; Al-Ghais, 2013; Ahmad *et al.,* 2016a; Hayat *et al.,* 2017). Each toxicant elicits a response from the biomarker, thus generating the idea of manipulating these biomaterials to develop a sensitive biosensor. A biosensor utilises naturally occurring recognition components isolated from the environment and biological system through separation and purification processes, or is synthetically synthesised to enhance the function by increasing the sensitivity and stability of the component (Bohunicky and Mousa, 2011). A biosensor also gives convincing result, consumes cheaper cost and less time, and does not require skilful technician to handle the test, as compared to other analytical tests (Mascini and Tombelli, 2008; Tothill, 2009). A biosensor is designed as a compact and sensitive sensing device by the utilisation of biological components such as enzyme and antibodies, with physicochemical detection of an analyte (Mascini and Tombelli, 2008; Bănică, 2012; Ngoepe *et al.,* 2013). This device is also capable of generating full or semi-quantitative analytical information based on the output (Thévenot *et al.,* 2001). The development of biosensor technology is associated with the increasing development of water companies, manufacturing industries, agricultural activities, mining activities, and urbanisation, in which this situation has demanded high quality and toxicant-free biosensors (Tothill, 2001). Biosensors are widely applied in biomedical diagnostics such as for the detection of diseases (*e.g.* diabetes) and pregnancy (Yoo and Lee, 2010; Bohunicky and Mousa, 2011; Mach *et al.,* 2011). Biosensors are also utilised in the agricultural and food industries to detect the existence of allergens and pathogens in the products (Narsaiah *et al.,* 2012; Rigi *et al.,* 2013). In addition, biosensors have been developed to monitor environmental pollutions, such as silage effluent (Stephens *et al.,* 1997), pesticides (Arduini *et al.,* 2006; Viswanathan *et al.,* 2009), heavy metals (Long *et al.,* 2013; Shukor *et al.,* 2013), bacterial pathogens (Liao *et al.,* 2007), and chlorinated solvents (Bhattacharyya *et al.,* 2005). In summary, the application of biosensors as a diagnostic tool has been used in various fields of study.

*Cholinesterase-based biosensor for heavy metal detection*

Biomonitoring programmes using enzyme-based biosensor have been applied to assess toxicant level in food safety and the environment (Luque de Castro and Herrera 2003; Amine *et al.,* 2006). Heavy metal is one of the most abundant toxicants in our environment that are of concern, since acute or chronic exposure to this compound affects mortality. Thus, biosensor assays based on enzyme inhibition have been developed by various researchers to evaluate the toxicity level of heavy metals, such as the studies done by Lee and Lee (2002), Soldatkin *et al.* (2012), and Shukor *et al.* (2013). García Sánchez *et al.* (2003) reported that only a few types of enzyme are suitable to be a biosensor candidate for the detection of heavy metal contamination. Our review focuses on cholinesterase (ChE) studies as its considerable sensitivity and ability to detect multiple toxicants have a great deal of potential for biosensor development. *In vivo* and *in vitro* studies using ChE in various species

have been implemented to elucidate adverse effects of toxicants; these studies enhance the information about the effects of heavy metals on ChE, especially the possibility for biosensor kit development (Table 2). ChE such as acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) can be distinguished based on substrate and inhibitor specificity, kinetic properties, and distribution in tissue (Xiao *et al.,* 2010; Romani *et al.,* 2011; Yang *et al.,* 2013). Hsiao *et al.* (2004) mentioned that AChE plays a big role in fast hydrolysis of the neurotransmitter acetylcholine in the synaptic cleft, while Giacobini (2003) and Reid *et al.* (2013) reported BChE as a co-regulator in the absence of AChE. Another main function of BChE is as a defence mechanism which works as a detoxifying enzyme in the liver (Sparks *et al.,* 1999; Çokuğraş, 2003).

*Anti-cholinesterase effects*

The activity of ChE is related to the effect of nerve agents, also called anti-cholinesterase, such as carbamate and organophosphate. The principle of using cholinesterase as a biosensor is based on the evaluation of the decrease in its enzymatic activity, since it is inhibited by anti-cholinesterase (Pohanka, 2009). This means that the substrate acetylcholine is unable to be hydrolysed, resulting in a decrease of or no production of 2-nitrobenzoate-5-mercaptothiocholine and 5-thio-2-nitrobenzoate. This resulted in yellow colour production on both chromogens which can be measured at 405 nm (Aidil *et al.,* 2013). Carbamate and organophosphate bind at the active site of cholinesterase through carbamylation and phosphorylation, respectively (Fukuto, 1990; Rosenberry *et al.,* 2005). Heavy metals such as As (Patlolla and Tchounwou, 2005; Ali *et al.,* 2010), Ag (Shukor *et al.,* 2013), Cd (Devi and Fingerman, 1995), Cr (Elumalai *et al.,* 2002), Cu (Elumalai *et al.,* 2002; Howcroft *et al.,* 2011; Lima *et al.,* 2013; Sabullah *et al.,* 2013; Shukor *et al.,* 2013), Ni (Arduini *et al.,* 2005), Pb (Mat-Jais and Mohamed, 2000), and Zn (Mat-Jais and Mohamed, 2000; Diamantino *et al.,* 2003) are capable of inhibiting ChE activity either *in vitro* or *in vivo*. Other metal ions such as aluminium ($Al^{3+}$) and iron ($Fe^{2+}$) cause the reduction of ChE activity in rat and human plasma, respectively (Yellamma *et al.,* 2010; Karami *et al.,* 2010).

Metal ions that have a positive charge and facilitate enzyme-catalysed reactions are known as cofactors (Mones *et al.,* 2007). The toxic level of metal ion may affect enzyme activity because of structure alteration, inhibition, and protein denaturation, causing cellular disorder (Blackwell *et al.,* 1995). Tomlinson *et al.* (1981) noted two effects of heavy metal on ChE activity: (1) activation, such as by magnesium ion ($Mg^{2+}$), calcium ion ($Ca^{2+}$), manganese ion ($Mn^{2+}$), and sodium ion ($Na^{+}$); and (2) inactivation or inhibition, such as by $Zn^{2+}$, $Cd^{2+}$, $Hg^{2+}$, $Ni^{2+}$, $Cu^{2+}$, and $Pb^{2+}$. $Ca^{2+}$ and $Mg^{2+}$ have been demonstrated to reactivate ChE activity after being inhibited by $Cd^{2+}$ and $Zn^{2+}$ (Elkhashab, 2013). Romani *et al.* (2003) reported that *in vitro* exposition of Cu improved catalytic efficiency of ChE in the brain and muscle, while an *in vivo* test by Lima *et al.* (2013) showed a significant increase in ChE activity during the first two days the fish were exposed to 0.06 mg/L of Cu, and decreased after seven days of exposure. Other *in vitro* studies also proved Cu inhibition towards ChE activity (Shukor *et al.,* 2013; Sabullah *et al.,* 2013). The effects of Cu on sea snails have only been shown *in vitro* but not *in vivo* (Cunha *et al.,* 2007). These findings provide much information especially on the combination of *in vivo* and *in vitro* studies in the development of sensitive biosensor kits.

Previous studies have shown that heavy metals block substrate metabolism, either by directly binding at the active site of the enzyme or at the allosteric site, which causes conformational change and substrate inability to form enzyme-substrate complex (Mathonet *et al.,* 2006; Giedroc and Arunkumara, 2007; Ahmad *et al.,* 2016b). Amino acid residue plays an important role in attracting the substrate or possible inhibitor to bind at the active or allosteric site of the protein (Glusker *et al.,* 1999; Armentrout *et al.,* 2013). The ChE inhibitor organophosphate has the affinity to bind at the esteratic site, which contains a serine (Ser) residue (Sultatos and Kaushik, 2008), while carbamate interacts with both esteratic and anionic subsites containing histidine (His), tyrosine (Tyr), and glutamate (Glu), or substitution by aspartate (Asp) (Nair and Hunter, 2004; Carolan *et al.,* 2010; Pohanka, 2011). Frasco *et al.* (2007) reported the irreversible inhibition of Hg through binding at histidine (His), methionine (Met), tryptophan (Trp), threonine (Thr), and asparagine (Asn) residues. Cu, Ni, and Zn have been reported to be capable of binding at the imidazole group of His through strong cation-π attraction (Bhanumathy and Balasubramanian, 1998; Rajesh, 2009; Ralph *et al.,* 2011; Rodzik *et al.,* 2020).

However, there is still no report on the interaction of other heavy metals with amino acids of ChE, although several studies have proven their inhibitory effects. Other possible theories of enzyme inhibition have arisen, such as the covalent binding of metal ion with carbon to form metal-organic compounds or binding at the sulfhydryl or thiol group of amino acids (Van Assche and Clijsters, 1990; Flora and Pachauri, 2010). Sarkarati *et al.* (1999) reported that the negative charge of amino acids such as glutamic and aspartic acids may

Table 2. The literature reports of analysis by ChE from various sources for toxicant detection.

| Source | Type of experiment | Detection | Reference |
|---|---|---|---|
| AChE (*Tilapia mossambica*) | Fish were exposed to sewage water of Ras Al Khaimah Emirate of the UAE (group/sewage, 164 ± 22 g). | 26.61 and 30.32% decrease of AChE activity in liver and muscle, respectively. | Al-Ghais (2013) |
| Brain and muscle AChE (*Prochilodus lineatus*) | Juvenile fish were acutely exposed to 1 and 5 mg/L of glyphosate-based herbicide or only water (control) for 6, 24, and 96 h. | Brain: AChE activity decreased to 11.75 and 17.88% after 96 h exposure to 1 and 5 mg/L of glyphosate-based herbicide, respectively, compared to the control.<br>Muscle: AChE was significantly reduced by 21.25% in fish exposed to 5 mg/L of glyphosate-based herbicide for 96 h. | Modesto and Martinez (2010) |
| Gill and liver AChE and BChE (*Carcinus maenas*) | *In vivo* effect of 3.12 µg/L concentration of chlorpyrifos-ethyl after 24 and 48 h exposure. | AChE: 24 and 48 h exposure caused 41.03 and 47.20% inhibition in hepatopancreas, and 36.08 and 27.79% in gills, respectively, compared to the control.<br>BChE: 24 h exposure caused 40.94 and 73.34% inhibition in hepatopancreas and gills, respectively, compared to the control. | Ghedira *et al.* (2009) |
| | *In vivo* effect of 5% of secondary treated industrial/urban effluent (STIUE) after 48 h exposure. | Gill and liver AChE were significantly inhibited by 5% of STIUE while both BChE showed no significant difference compared to control. | |
| Brain and serum AChE and serum BChE (*Cyprinus carpio*, *Abramis brama*, *A. ballerus*, *Blicca bjoerkna*, *Rutilus rutilus*, *Alburnus alburnus*, *Leuciscus idus*, *Perca fluviatilis*, *Stizostedion lucioperca*, *Esox lucius*, and *Coregonus albula*) | *In vitro* effect of DDVP. | Brain and serum AChE of *Leuciscus idus, Esox lucius*, and *Alburnus alburnus* exhibited the highest $K_{II}$ value at 51.7 ± 6.8, 17.7 ± 8, 2.5 ± 0.1 × $10^3$ $mol^{-1}$ $min^{-1}$, respectively. | Chuiko (2000) |
| Plasma BChE (*Trichechus manatus*) | Blood sample of captive manatees at Pernambuco, Alagoas, and Paraíba (Brazil). | 40% inhibition of BChE activity in samples from Alagoas and Paraíba. | Anzolin *et al.* (2012) |
| AChE (purified *Bactrocera dorsalis* head) | *In vitro* effect of purified AChE with different concentrations of eserine, BW284C51, and ethopropazine. | $IC_{50}$ values: (2.80 ± 0.57) × $10^{-8}$ M, (8.59 ± 1.67) × $10^{-8}$ M, and (1.17 ± 0.65) × $10^{-5}$ M for eserine, BW284C51, and ethopropazine, respectively. | Hsiao *et al.* (2004) |
| BChE (human serum) | Investigation of the inhibition behaviour of $Hg^{2+}$. | $EC_{50}$ value at 1.2 × $10^{-6}$ M and the type of inhibition was non-competitive with $K_i$ value of 0.12 µM. | Mahmod (2001) |
| BChE (blood sample of *Leporinus macrocephalus*) | Purified BChE test with selected anti-cholinesterase in the presence of 2 mM BSCho substrate. | $IC_{50}$ values of chlorpyrifos oxon, methyl paraoxon, iso-OMPA, physostigmine, BW284C51, and procainamide were 2.2 × $10^{-12}$, 1.7 × $10^{-10}$, 5 × $10^{-10}$, 4.7 × $10^{-9}$, 1.8 × $10^{-4}$, and 8 × $10^{-3}$ M, respectively. | Salles *et al.* (2006) |

| | | | |
|---|---|---|---|
| AChE (*Electrophorus electricus*) | Commercial AChE *in vitro* test with surfactants. | AChE sensitivity to benzalkonium chloride, sodium dodecyl sulphate, and hexadecylpyridinium bromide at the concentrations of 0.35 mg/L, 2.5 µM, and 2.5 µM based on optimal procedure, respectively. | Kucherenko *et al.* (2012) |
| Brain AChE (*Periophthalmodon schlosseri*) | Purified AChE incubated in 1 mg/L of selected metal ions, then continued with $IC_{50}$ determination. | Cu, Hg, Cr, and As showed significant inhibition compared to control with $IC_{50}$ values of 0.088, 0.371, 0.112, and 0.141 mg/L, respectively. | Sabullah *et al.* (2013) |
| Brain AChE (*Colossoma macropomum*) | Crude AChE incubated with different concentrations of selected carbamate. | $IC_{50}$ values of carbaryl and carbofuran were 0.45 and 0.95 µmol/L, respectively. | Assis *et al.* (2010) |
| AChE (*Electrophorus electricus*) | Commercial AChE *in vitro* test with selected heavy metals at the final concentration of 5 mg/L. | Cu, Ag, and Hg showed more than 50% inhibition with $IC_{50}$ values of 1.212, 0.1185, and 0.097 mg/L, respectively. | Shukor *et al.* (2013) |
| AChE (*Pomatoschistus microps* head) | Fish were exposed to different concentrations of Cu (0 to 400 µg/L) and Hg (0 to 50 µg/L). | Cu and Hg exposure with concentrations of 200 and 25 µg/L, respectively, significantly inhibited more than 50% of activity. $EC_{50}$ values of Cu and Hg were 385.9 and 93.57 µg/L, respectively. | Vieira *et al.* (2009) |
| ChE (*Enchytraeus albidus*) | *In vivo* and *in vitro* test on soil properties, copper, and phenmedipham. | ChE activity significantly decreased after 3 weeks of exposure to Cu (320 mg/kg) and phenmedipham (32 mg/kg). $IC_{50}$ values of Cu and phenmedipham were 0.012 and 15.5 µM, respectively. | Howcroft *et al.* (2011) |
| Haemolymph AChE (*Carcinus maenas*) | The effect of Cu, Cr, and a mixture of these metals on AChE. | The activity decreased to 75.20 ± 4.51 and 43.83 ± 4.41 U $mg^{-1}$ after exposure to Cu and Cr at concentrations of 15 and 10 µg/L, respectively, and the mixture of Cu and Cr reduced AChE activity to 33.05 ± 2.78 U $mg^{-1}$ (Control: 134.02 ± 7.25 U $mg^{-1}$). | Elumalai *et al.* (2002) |
| Brain AChE (*Channa striatus*) | *In vitro* effect of Hg, Cd, Pb, Ni, and Zn. | Based on a concentration of 20 ppm, Hg inhibited 180.76 ± 25.94%; Cd, 148.08 ± 5.36%; Pb, 88.19 ± 1.19%; Zn, 62.43 ± 2.28%; and Ni, 64.19 ± 2.38%. | Mat-Jais and Mohamed (2000) |
| AChE (bovine erythrocytes) | Amperometric measurement of enzyme activity treated with $Cu^{2+}$, $Cd^{2+}$, $Fe^{3+}$, and $Mn^{2+}$. | All the metal ions showed reversible non-competitive inhibition behaviour with the Ki values of $2.72 \times 10^{-4}$ (Cu), $3.41 \times 10^{-4}$ (Cd), $6.84 \times 10^{-4}$ (Mn), and $14.71 \times 10^{-4}$ (Fe) mol/L. | Stoytcheva (2002) |
| BChE (human serum) | The effects of $Cd^{2+}$, $Zn^{2+}$, and $Al^{3+}$ on purified BChE. | $K_i$ values of Cd, Zn, and Al were 0.004, 0.009, and 0.312 mM, respectively. | Sarkarati *et al.* (1999) |
| cDNA AChE (*D. melanogaster*) | Purified AChE was incubated with metal concentrations of 0.1, 1.0, and 10 mM in different buffer solutions (phosphate and Tris buffer). | All tested heavy metals inhibited more enzyme activity in Tris buffer; 1 and 10 mM of Cu, Zn, and Cd inhibited 50% of AChE activity. | Frasco *et al.* (2005) |

$EC_{50}$: Effective concentration that causes 50% response, and $IC_{50}$: concentration that causes 50% inhibition.

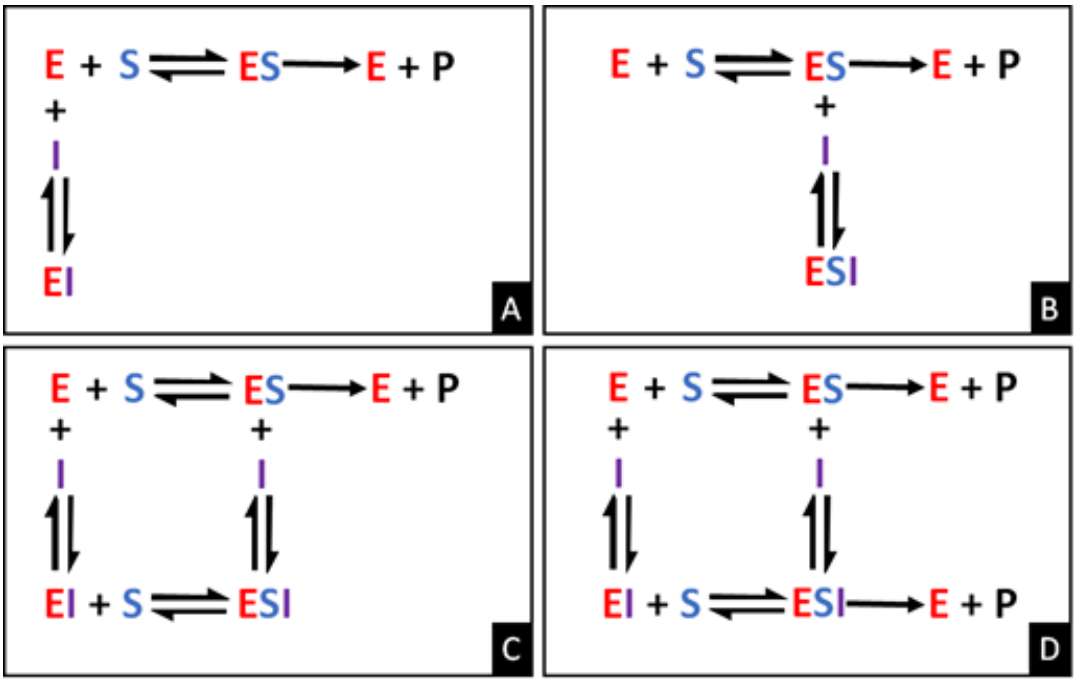


Figure 2. Mechanism of reversible inhibition of ChE by a metal ion. E = ChE, S = acetylcholine substrate, I = metal ion which is ChE inhibitor, and P = product of reaction (choline and acetate). (A) Competitive inhibition where either ChE reversibly interacts with acetylcholine to form ES complex followed by product formation, or ChE is reversibly inhibited by a metal ion. (B) Uncompetitive inhibition where metal ion only reversibly inhibits the reaction after the formation of ES complex. (C) Non-competitive inhibition shows the metal ion's ability to form ESI complex either by directly interacting with ChE or ES complex. Acetylcholine also shows the same interaction with metal ion but is only hydrolysed after complete formation of ES complex. (D) Mixed-type inhibition is similar to non-competitive inhibition in which acetylcholine is capable of being hydrolysed during the formation of ES or ESI complex.

interact with the metal ion, especially at the amino acid side chain that contains a carboxyl group. Another possibility is that the heavy metals attract the other side chain of Met (thioether), Ser, Thr, Tyr (hydroxyl groups), Asp, and Gln (carbonyl group) (Glusker *et al.,* 1999; Armentrout et al., 2013). Frasco *et al.* (2007) investigated the inhibition of ChE by heavy metals through kinetic studies, X-ray crystallography, and dynamic light scattering. Assis *et al.* (2015) determined the inhibitory effect of metal ion in *Rachycentron canadum* (black kingfish) brain ChE through a kinetic study, where $Hg^{2+}$ was competitively inhibiting the enzyme activity. In contrast, Frasco *et al.* (2007) showed irreversible inhibition in ChE activity: $Cu^{2+}$ and $Cd^{2+}$ showed non-competitive inhibition while $Zn^{2+}$, Pb2+, and $As^{5+}$ displayed mixed-type inhibition. Similar results were reported by Sabullah *et al.* (2015) and Vivek *et al.* (2016) on $Cu^{2+}$ and $Cd^{2+}$, which non-competitively inhibited ChE in *Puntius javanicus* (Java barb) and male albino rat, respectively. $Pb^{2+}$ displayed uncompetitive inhibition of ChE in human erythrocytes (Gupta *et al.,* 2015). Figure 2 illustrates the main types of metal ion inhibition on ChE activity.

## Conclusion

The application of the ChE inhibition test has become a significant interest in the development of a sensitive biosensor kit for toxicant detection. At present, ChE has been reported to be sensitive towards heavy metals either *in vivo* or *in vitro*. Hence, it will become a beneficial tool for real-time analysis in the future for multiple-toxicant detection in various industrial sectors such as food safety and medicine.

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