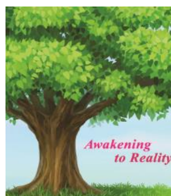

Available online at www.elixirpublishers.com (Elixir International Journal)

Applied Chemistry

Elixir Applied Chemistry 160 (2021) 55724-55729

Elixir ISSN: 2229-712X

Development of a Peroxidase-Based Assay for Assessment of Heavy Metals in Aqueous Samples

A.M. Magomya, U.C. Okpaegbe, G.G. Yebpella, B.N. Hikon and Y. Joshua
Department of chemical sciences, Federal University, Wukari, Nigeria

ARTICLE INFO

Article history:
Received: 2 October 2021;
Received in revised form: 1 November 2021;
Accepted: 9 November 2021;

Keywords
Peroxidase,
Ipomea Batata,
Guaiacol,
Heavy Metals,
Inhibition,
Toxicity Evaluation.

ABSTRACT

The assessment of heavy metals (Hg, Cu, Pb and Cd) by means of a Peroxidase - based assay is here presented. The method is based the inhibition of peroxidase activity by heavy metals. Measurements were made spectrophotometrically via the catalytic oxidation of guaiacol in the presence of H_2O_2. Preliminary characterization and optimization of the assay system revealed enzyme activity of 130 U/ml, Vmax of 0.178mM/min and Km of 0.184mM. Optimal reaction time was found to be 7 minutes; optimal pH was 6 and temperature was 40°C. Inhibition-based estimation of heavy metal ions was evaluated via calibration curves of metal concentration against %inhibition of peroxidase activity. The assay revealed detection limits between 0.006 – 0.045mg/L for the metals and repeatability in the range of 2.3 – 5.4% RSD. Application of the method to water analysis revealed percentage recoveries between 76.0 – 124.0%. Overall, the method showed good potential for further use in the biomonitoring of heavy metal pollutants.

© 2021 Elixir All rights reserved.

Introduction

With increased worldwide industrialization over the last 25 years, both developed and developing nations face increased environmental problems due to the release of toxic chemicals into aquatic environments. Today, enormous pressure is being put on researchers to evaluate thousands of chemicals that are released into the environment on daily basis. Heavy metals are among the toxic substances that have been shown to be responsible for many ecological and human health problems[1]. In recent years there has been an increased ecological and global health concern associated with environmental contamination by these metals[2]. Human exposure to heavy metals has risen dramatically as a result of an exponential increase in their use in several industrial, agricultural, domestic and technological applications.

Enzyme inhibition assays have the potential to rapidly screen and identify heavy metals in environmental samples. Such assays are particularly advantageous because of their high sensitivity, low cost, simplicity and less time requirement. These factors make the methods more convenient for in-*situ* screening and real time analysis. In addition, enzyme–based methods have the ability to determine bio-available heavy metals and also evaluate the toxicity of samples. In environmental pollution monitoring, it is becoming a general opinion that chemical analysis by itself does not provide sufficient information to assess the ecological risk of pollutants. The best scenario for routine bio-monitoring of heavy metals has been said to be the marriage between instrument- and bioassays. In view of this, there is need for more emphasis on the development of bioassays and related procedures to be used in water quality monitoring.

Various enzymes such as acetyl cholinesterase [4,5] alkaline phosphatase [4] urease [6] glucose oxidase [7] in assays and biosensors for the determination heavy metals. The most common approach to enzymatic determination of heavy metals is based on the inhibition of specific catalytic activities. The presence of low concentrations of heavy metals is known to strongly affect enzyme activity. Therefore, by measuring enzyme activity in the presence and absence of the metals, their concentrations be can be evaluated

In this study, the Inhibition of crude peroxidase from sweet potato (*Ipomea batata*) has been examined as a method for assessing heavy metal levels in aqueous sample. The assay is based on the spectrophotometric measurement of the inhibitory effects of heavy metals on peroxidase activity. Experimental parameters such as pH, temperature, reaction time and contact time with metals have been characterized and optimized. This is the first report on the use of peroxidase from sweet potatoes in an enzyme inhibition-based assay for evaluation of heavy metal ions.

Materials and Methods

Materials

Sweet potato tubers were purchased from a local market in Taraba state Nigeria. Disodium hydrogen phosphate and Sodium dihydrogen phosphate were from BDH, H_2O_2 was from Merck, South Africa and Guaiacol was from Loba Chemie, India. The standards for Copper (Cu^{2+}) cadmium (Cd^{2+}), lead (Pb^{2+}) and mercury (Hg^{2+}) were purchased as atomic absorption standard solutions (1000 mg L^{-1} AAS) from Sigma Aldrich. All chemicals used were of analytical grade.

Extraction of Crude Peroxidase from *I. batata tubers*

The method of Eze *et al.* [10] was used with slight modifications. A piece of a potato tuber was washed, peeled and diced into tiny pieces. Twenty (20) grams of the diced potatoes was ground till smooth. It was then added to 100 mL of phosphate buffer (0.1M, pH 7), and homogenized

Tele:
E-mail address: amagomya26@gmail.com

© 2021 Elixir All rights reserved

thoroughly using a blender until no lumps are observed. The homogenate was filtered using four layers of cheesecloth and the filtrate was centrifuged at 4,000 rpm for 30 min at room temperature. The supernatant was collected as crude Peroxidase extract and stored at 4^oC

Determination of Peroxidase Activity

Peroxidase activity in the extract was measured using guaiacol as substrate;1 mL of 0.5% H_2O_2 was added to 1ml of 6% guaiacol. The reaction was initiated by adding 1ml of the crude peroxidase extract. The change in the absorbance was monitored at intervals of 1 minute for 5 minutes on a Uv-Vis spectrophotometer (T60) at 470nm. A blank solution was prepared in the same way as the test mixture but with 1ml buffer in place of the enzyme solution. The blank absorbance was also taken at 470 nm and appropriate corrections were applied. Peroxidase activity was estimated using the equation:

$$\text{Peroxidase Activity (U/L)} = \frac{\Delta A(\text{sample}) \times \text{Total assay Volume}}{\Delta T \times \varepsilon \, l \times \text{Volume of enzyme}}$$

Where:

A (sample) = measured absorbance of test Sample against the blank

n = dilution factor

ΔT = Change in time

ε= extinction coefficient (Guaiacol)

l = Cuvette diameter (path length)

The activity was calculated in units per L, one unit (U) of activity is the amount of enzyme extract that causes the oxidation of 1 µmol of guaiacol per minute, under the stated working conditions.

Determination of Kinetic Parameters (*Km & Vmax*)

The kinetic parameters (*Km and Vmax*) of peroxidase were evaluated by means of the Michealis -Menten model. Peroxidase activity was measured with varying concentrations of substrate (guaiacol) while keeping the enzyme concentration constant. The Michaelis-menten graph of enzyme activity (µmol product formed per min) against substrate concentration was plotted using Prism-Graphpad software. The *Vmax* (maximum velocity achieved by the system, at saturating substrate concentrations) and K_m (substrate concentration at 50% of the *Vmax*) were estimated from the plot.

Effect of Substrate Concentration on Peroxidase Activity

Different concentrations of substrate solution ranging from 0.5 to 6% were tested for peroxidase activity as previously described. The resulting absorbances were recorded on a UV-Vis spectrophotometer at 470nm.

Effect of pH on Peroxidase Activity

The effect of pH on peroxidase activity was investigated with a view to establishing the optimal working pH. The assay mixture contained 1ml H_2O_2 (0.5%), I ml Guaiacol (6%) and 1ml enzyme extract. pH adjustments (6.0 – 9.0) were made with 0.1M NaOH and 0.1M HCL while keeping all other parameters constant. Absorbance of assay activity was taken at 5 min on a UV-Vis spectrophotometer.

Effect of Temperature on Peroxidase Activity

The effect of temperature on peroxidase activity was determined over a temperature range of 30°C to 70°C; 1 ml of crude enzyme in a test tube was placed in a thermostated water bath and the temperature monitored with a thermometer. The enzyme solutions were removed after attaining the required temperature and the peroxidase activity was tested as previously described.

Effect of Reaction Time

The effect of time on assay activity was evaluated by taking measurements of the assay absorbance at intervals of 1 minute for a period of 10 minutes.

Effect of Contact Time with Heavy Metal ions

The effect of contact time on peroxidase inhibition efficiency were tested with the heavy metal ions Cu^{2+}, Cd^{2+}, Hg^{2+} and Pb^{2+}. The respective metal solutions (1ml of 1mg/L concentration) were pre-incubated with the enzyme extract at different time durations ranging from 2–10 minutes. The peroxidase activity was then measured as previously described.

Inhibition Assay for Heavy Metals

Standard solutions (0.1, 0.5, 1.0, 1.5, 2.0 mg/L) of the heavy metals Cu^{2+}, Cd^{2+}, Hg^{2+}, and Pb^{2+}were prepared by serial dilution from the respective 1000 ppm stock solution. The inhibitory effects of the metals were tested on the extracted peroxidase. For each measurement, 1ml metal ion solution and 1ml enzyme extract solution were pre incubated for 5 mins followed by addition of 1ml of H_2O_2 (0.5%) and 1 ml Guaiacol . The absorbance of the resulting solution at 5 minutes was read against a blank at 470nm on a uv-vis spectrophotometer. The level of inhibition for each tested metal concentration was obtained using the relationship:

$$\% \text{ inhibition} = \frac{Ao - Ai}{Ao} \times 100$$

where;

Ao = the obtained absorbance without the inhibitor (metal ion)

Ai = absorbance obtained after pre- incubation with metal ion.

A graph of % inhibition against concentration was plotted for each heavy metal to obtain the linear range for estimation of the metal concentration.

Results and Discussions

Peroxidase Activity and Kinetic characteristics

The peroxidase (POD) used in this study was extracted from sweet potato (*Ipomea batata*) tubers. POD activity was estimated via the oxidation of guaiacol (ε = 26.6 mm^{-1} cm^{-1}) in the presence of H_2O_2.

Peroxidase catalyses the reaction: $2H_2O_2 \xrightarrow{peroxidase} 2\,H_2O + O_2$

The oxygen produced then reacts with Guaiacol (2-methyoxyphenol) to produce a brown product, tetraguaiacol (oxidized Guaiacol). The rate of formation of tetraguaiacol which is a measure of POD activity was assayed with a UV spectrophotometer at 470 nm

$$\text{Guaiacol} + 2H_2O_2 \xrightarrow{peroxidase} \text{Tetraguaiacol (brown colour)}$$

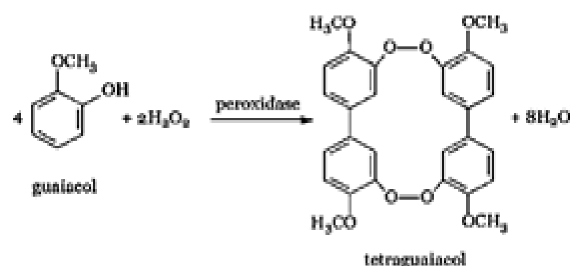

Figure 1. Enzymatic Conversion of guaiacol to tetraguaiacol in the presence of hydrogen peroxide

The Guaiacol-peroxidase activity of Ipomea *batata* crude extract was found to be 132 U/ml (Table 1). A look at peroxidases from other plant sources revealed wide variations in activity. Values ranging from 0.0096 U/ml for *I.batata* [11] to 10,585U/ml for lettuce stems [12] have been reported in literature. The reasons for these variations can be attributed to

the nature of substrates used, purity of enzymes, extraction methods and experimental conditions. POD kinetics was investigated through the Michaelis–Menten model with guaiacol used as variable substrate and H_2O_2 at saturated concentration. The plot demonstrates an enzyme's affinity for a substrate, represented by Km, and the maximum catalytic velocity, represented by Vmax. The values for *Km* and *V*max were 0.184mM and 0.178mM/min respectively (Table 1). In general, a low Km value indicates a high affinity between the enzyme and substrate thus less of the substrate is necessary to reach saturation. The km value obtained in this study is relatively low thus suggests good affinity of the peroxidase for the substrate under the test conditions.

Table 1. Characteristics of Crude peroxidase from *Ipomea batata*

Parameter	Value
Enzyme activity	130 U/ml
Vmax	0.178mM/min
Km	0.184mM

Optimization of Substrate concentration

Figure 1 shows the effect of substrate (Guaiacol) concentration on peroxidase assay activity. Enzymes generally follow saturation kinetics with respect to the substrate. It is believed that the substrate occupies specific active sites on the enzyme to be properly oriented for a round of reaction [13]. As the substrate concentration increases, all the active sites might be occupied and any further increase in substrate concentration does not affect the rate of reaction. In this study the optimal substrate concentration was found to be 6%. Above this concentration, a slight decline in activity was observed.

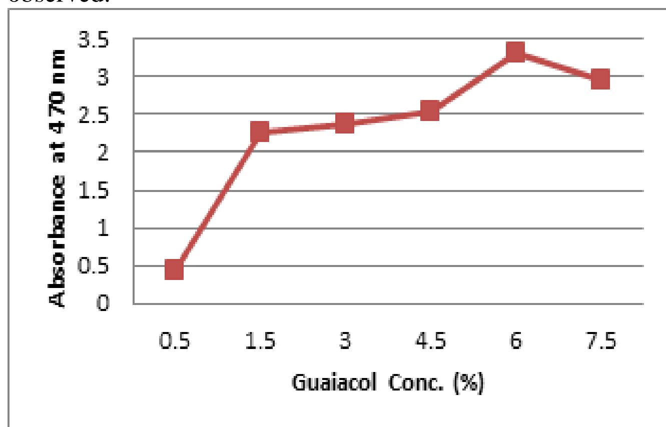

Figure 1. Optimization of substrate concentration for Peroxidase assay

Optimization of Reaction Time

The longer an enzyme is incubated with its substrate, the greater the amount of product that will be formed. However, the rate of formation of product is not a simple linear function of the time of incubation. Figure 2 shows the effect of reaction time on the activity of *I. Batata* POD. As can be seen, assay activity increased steadily from 1-7 minutes after which it began to decline. Enzyme catalysed reactions are reversible therefore, when there is little or no product present the reaction proceeds only in the forward direction. However, as the reaction continues, there is a significant accumulation of product hence a significant rate of back reaction. As a result, the rate of formation of product slows down as the incubation proceeds, and if the incubation time is too long, then the measured activity of the enzyme becomes low. This trend is likely the cause of the observed decline in POD activity from 8 minutes (Fig. 2). Although the optimum assay activity was observed at 7 minutes, 3 minutes reaction time was adopted for this assay. This was considered sufficient because over 90% of the activity had been established at that time.

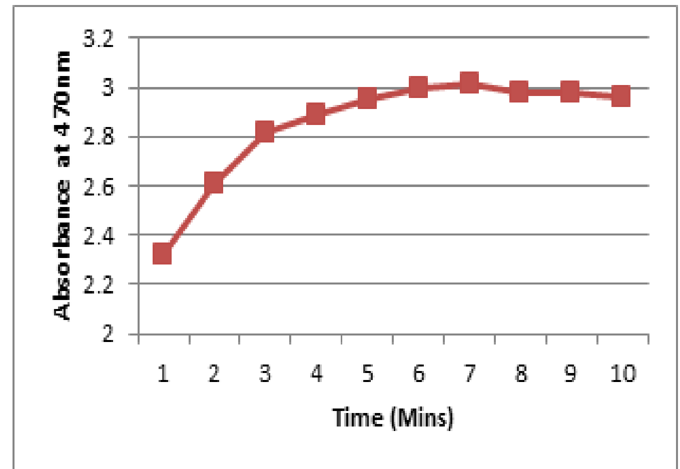

Figure 2. Optimization of reaction time for peroxidase assay

Optimization of pH

The effect of pH on POD activity as investigated over a pH range of 5 to 10 is shown in Figure 3. pH is a crucial factor in all enzyme assays because it is known to affect the ionization state of enzymes. If the state of ionization of amino acids in a protein is altered, the ionic bonds that help to determine the 3-D shape of the protein would be affected and this can lead to altered protein recognition or inactivation of enzymes. Changes in pH may not only affect the shape of an enzyme but may also change the shape or charge properties of the substrate so that either the substrate cannot bind to the active site or it cannot undergo catalysis. Maximum activity for the assay in this study was recorded at pH 6 after which a sharp decline in activity was observed. Our findings here indicate that *Ipomea batata* POD is more active in the acidic region than in the alkaline. Similar findings have been reported for different plant PODs in literature; The optimum pH for POD from *Carica papaya* was found to be 4.6 [15], maximum activity for *Rosmarinus officinalis L.* Leaves was reported at 6.0 pH [16] and for oil palm 5.0 [17]. Similarly pH optima for silk peroxidase was observed at 6.5[18] and for *S. melongena* at 5.5 [19]. In general, the pH optima for POD is said to range from acidic to slightly alkaline region (4.0-8.0). Our findings in the current study agree with this.

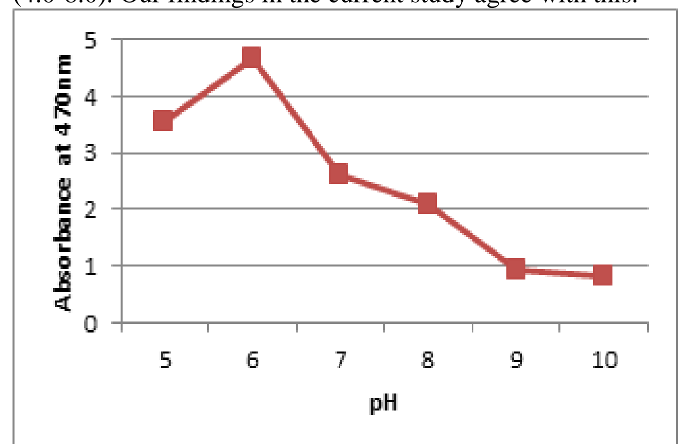

Figure 3.Optimization of pH for peroxidase assay.

Optimization of Temperature

The dependence of POD activity on temperature is presented in Figure 4. As with many chemical reactions, the rate of an enzyme-catalysed reaction increases as the temperature increases. However, at high temperatures the rate

decreases again because the enzyme becomes denatured and can no longer function. In this study optimum assay activity was recorded at 40°C. A 17 % decline in activity was observed at 50°C and then a 21% decline at 60°C. The findings here indicate that 79% of POD activity was retained at 60°C. Peroxidases are generally known to exhibit high thermal stability [20]. The optimal activities of peroxidases from four bean varieties (*Vigna unguiculata*, *Vigna subterranean, Phaseolus vulgaris* and *Glycine max*)) were recorded between 40 and 50°C. It was also observed that they all retained over 60% of activity at 60°C [21]. Similarly The optimum temperature of the enzyme from three sources (*Achyranthus aspera*, *Ricinus communis* & *Calotropis procera)* was found to range from 40°C to 60°C [22] similarly, Peroxidases isolated from sunflower roots [23] and Lettuce stems[12] were reported to show maximum activities at 40°C, and 50°C respectively. The temperature optima we report in this study is within the range of values that have been reported for other plant peroxidases. The enzyme also shows high thermal stability in accordance with what have been previously reported for other plant peroxidases.

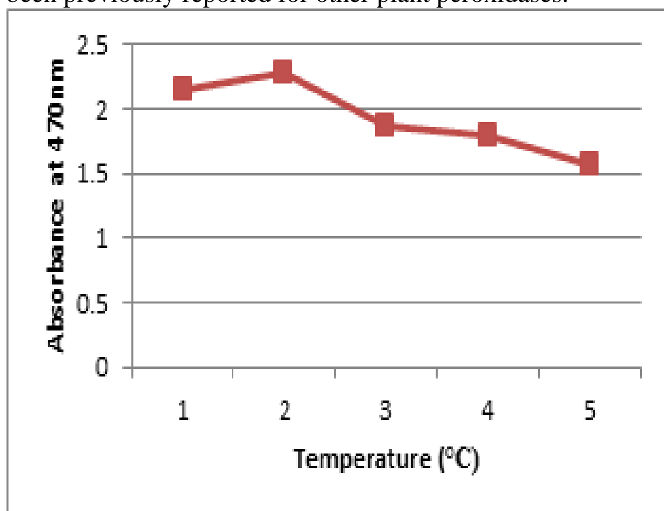

Figure 4.Optimization of Temperature for peroxidase assay

Optimization of Contact Time with Heavy Metal Ions

Figure 4 shows the dependence of inhibition efficiency on incubation (contact) time as investigated for all the heavy metals (Hg, Cu, Pb and Cd) at 1 mg/L concentration. The percentage of inhibition increased with incubation time for all the investigated metals. Overall, Hg seemed to show higher inhibitory strength followed by Cd then Pb and Cu respectively. The reason for increased inhibition with time is evidently because the longer the time, the more the interaction between inhibitor and enzyme. When the incubation time is insufficient, the enzymatic activity is not fully inhibited and low concentrations of the inhibitors may not be detected. On the other hand, a long exposure time to the inhibitor solution may dramatically damage the structure and the properties of the enzyme [24]. The incubation time must therefore be sufficient to give an appreciable inhibition but not too much to result in damage to enzyme structure. A longer incubation time also means a longer analysis time. 4 minutes incubation time was selected for this study. This was chosen as a compromise between the maximum percentage inhibition and analysis time. Also, the inhibition levels obtained after 4 minutes incubation time is considered sufficient because it is up to 80% of the maximum obtainable inhibition. This is usually taken as an informative indicator of sufficient inhibition [25]

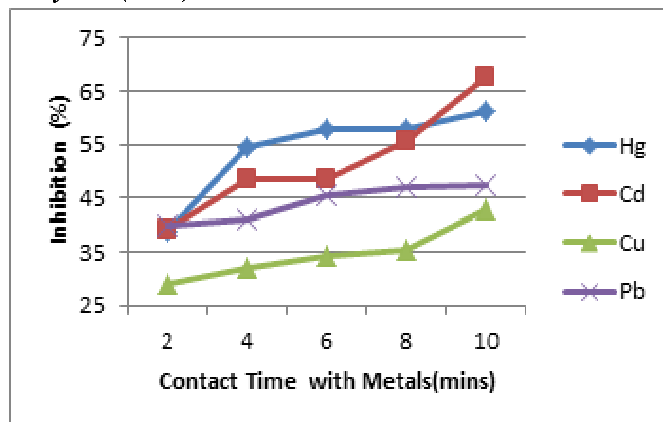

Figure 5. Optimization of Contact Time with Heavy metals

Inhibition-based Determination of Heavy Metals in Aqueous Samples

The principle of enzyme-based determination of heavy metals is premised on the interaction of metals with the sulfhydryl group on most enzymes to form metal sulphides. This bond formation results in the inhibition of enzymatic activity and measuring the level of inhibition offers an indirect method of estimating the metal (inhibitor) concentration. Evaluation of the inhibitive responses of the heavy metals ions (Pb^{2+}, Hg^{2+}, Cd^{2+} and Cu^{2+}) on peroxidase activity revealed a dose dependent relationship between metal concentration and % inhibition of activity. Figure 6 shows the inhibition calibration plots for the four metals evaluated.

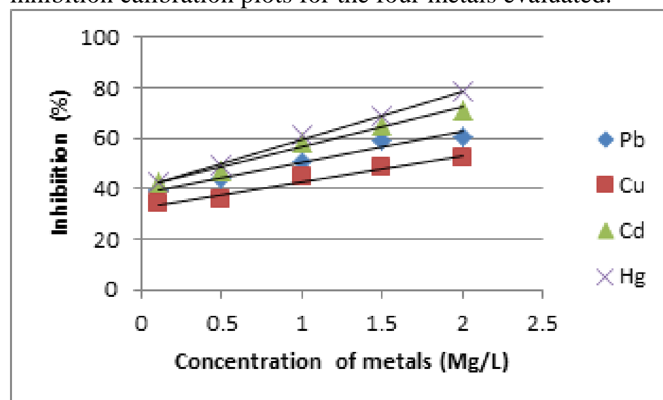

Figure 6. Calibration Plots for the determination of (Pb^{2+}, Cu^{2+}, Cd^{2+} and Hg^{2+}).

As can be seen, the inhibition responses vary for each of the investigated metals. For the concentration range of 0.1 – 2.0mg/L considered in this study, the inhibition range for Pb was from 39.19 – 60.81 %, Cu was from 33.89 – 52.23%, Cd was 42.32 – 71.30 and Hg was 42.80 – 78.50%. The IC 50 values for the metals (Table 2) were obtained as 0.97mg/L, 1.69mg/L 0.593mg/L and 0.483 mg/L for Pb, Cd, Cu and Hg respectively. IC 50 refers to the concentration that causes 50% inhibition of activity. It is an important parameter in bio-monitoring and toxicity evaluation of pollutants. From the obtained values in this study, the toxicity strengths of the investigated heavy metals can be said to be in the order Hg(II)>Cd(II) > Pb(II)> Cu(II). This trend is in agreement with the WHO toxicity trend for the metals when considered on the basis of their maximum permissible limits (MPL) in drinking water which has been given as 0.001 mg/L for Hg, 0.005mg/L for Cd, 0.05mg/L for Pb and 1.0mg/L for Cu [26].

The analytical characteristics of the assay calibration Curves (Table 2) indicate good linear correlation (96 -99%) within the studied concentration range. The detection limits

for the method were found to be 0.006mg/L, 0.045mg/L, 0.022mg/L and 0.005mg/L for Pb, Cu, Cd and Hg respectively. Again, when assessed on the basis of the MPL for drinking water, it can be seen that the method can detect Pb Cu and Cd at concentrations even lower than their MPLs. However for Hg, standard addition method may be required to achieve determination of its MPL with respect to drinking water. Repeatability of the assay (Table 2) ranged from 2.3 to 5.4% (RSD) for the determinations of 1.00mg L^{-1} metal concentration ($n = 6$). Generally in chemical analysis, a repeatability of less than 5% for 6 measurements at mg/L concentration is considered reliable. On this basis, the repeatability of the current assay is considered good.

Table 2. Analytical characteristics of Peroxidase-based assay for determination Heavy

	Pb^{2+}	Cu^{2+}	Cd^{2+}	Hg^{2+}
Equation for calibration graph	y=12.14x + 38.23	y=10.38x + 32.45	y=15.75x + 40.65	y=18.92x +40.86
Correlation coefficient	0.975	0.96	0.989	0.99
Repeatability (% RSD)	2.3	3.6	5.4	2.8
Detection Limit ($mg.L^{-1}$)	0.006	0.045	0.022	0.005
IC50 ($mg.L^{-1}$)	0.97	1.692	0.593	0.483

The feasibility of the assay towards the determination of heavy metals was examined by carrying out recovery tests for the metals in tap water spiked with known amounts of the metals. The results which are presented in table 3 showed recoveries in the range of 76% - 124%. This shows good potential of the method in practical applications however, the over 100% recovery recorded for some of the samples could be an indication of the presence of other metal pollutants.

Table 3. Recovery Tests for Heavy metals (Pb^{2+}, Cu^{2+} Cd^{2+} and Hg^{2+}) in Tap water.

Heavy metal ion	Added ($mg{\cdot}L^{-1}$)	Found ($mg{\cdot}L^{-1}$)	Recovery (%)
Pb^{2+}	0.25 1.75	0.31 1.68	124 96
Cu^{2+}	0.25 1.75	0.29 1.52	116 87
Cd^{2+}	0.25 1.75	0.17 2.05	76 117
Hg^{2+}	0.25 1.75	0.22 1.85	88 105

Conclusion

This study highlights the potential of peroxidase from *Ipomea batata* as an enzyme that can be used for the assessment of heavy metals in water samples. All the metals investigated showed significant responses to the enzyme even at low concentrations. This indicates high sensitivity of the assay system towards heavy metal determination. The method also showed good repeatability and stability to high temperatures. It is important to note that that the use of enzyme assays in the area of environmental analysis is a great challenge because environmental samples are highly complex and some of the components can promote interferences such as inhibition or enhancement of the biological component. As a result, methods based on enzyme inhibition cannot alone cannot provide the concentration of a specific metal in a real sample matrix; In principle, an enzyme inhibition method would detect all contaminants endowed with the same biochemical effect. Therefore, the methods can best be used in complementing other analytical methods in the area of rapid monitoring and bio-assessment of heavy metal pollution. In addition, the method has the advantage of being environmentally friendly due to the use of an enzyme from a plant source thus providing cleaner analytical methods for minimizing the use chemical reagents.

References

[1] Argese, E., Bettiol, C., Marchetto, D., Vettori, S.D and Zambon, A., (2005). Study of the toxicity of phenolic and phenoxy herbicides using submitochondrial particles assay. *Toxicol. In Vitro,* 19, 1035-1043.

[2] Duffus J.H (2002) "Heavy Metals-A meaningless term? *Pure Appl.Chem*. 74, 793-807

[3] Shukor, M.Y., Baharom, N.A., Rahman, F.A., Abdullah, M.P.A.,Shamaan, N.A. and Syed M.A. (2006). Development of a heavy metals enzymatic-based assay using papain. *Analytica Chimica Acta,* 566, 283–289.

[4] Chouteau, C., Dzyadevych, S. V., Chovelon J.-M. and Durrieu,C. (2004). Development of novel conductometric biosensors based on immobilised whole cell Chlorella vulgaris microalgae. *Biosensors and Bioelectronics,* 19 (9),1089–1096.

[5] Carlo, M.D., M. Mascini, A. Pepe, G. Diletti and D. Compagnone, 2004. Screening of food samples for carbamate and organophosphate pesticide using an electrochemical bioassay. *Food Chem.,* 84, 651-656

[6] Magomya, A.M and. Barminas, J.T. (2019). A conductometric method for determination of arsenic ions (As^{3+}) based on inhibition of urease activity. *FUW Trends in Science & Technology Journal,* 2 (1B). 439 – 444

[7] Malitesta, C. and Guascito, M.R., (2005). Heavy metal determination by biosensors based on enzyme immobilised by electropolymerisation. *Biosens. Bioelectron.,* 20, 1643-1647

[8] Silwana, B., Charlton Van Der Horst, C. Emmanuel Iwuoha, E andVernon Somerset, V (2014). Amperometric determination of cadmium, lead, and mercury metal ions using a novel polymer immobilized horseradish peroxidase biosensor system . *J Environ Sci Health A Tox Hazard Subst Environ Eng.* 49(13), 1501-11

[9] Baskaran, G., Masdor, N.A., Syed, M.A and Shukor, M.Y. (2013). An inhibitive enzyme assay to detect mercury and zinc using protease from coriandrum sativum. The Scientific World Journal, Article 678356

[10] Eze, S.O.O., Chilaka, F.C., Nwanguma, B.C (2010). Studies on thermodynamics and kinetics of thermo inactivation of some quality-related enzymes in white yam (*Dioscorearotundata). J. Thermodyn. Cataly,* 1, 104-109.

[11] Bharadwaj, P.S (2019). Extraction and purification of peroxidase enzyme from sweet potato. The Pharma Innovation Journal 8(9), 418-422

[12] Yihong, H.U., Juan W. U., Ping L. U., and Yiwei, M. O. (2012). Purification and partial characterization of peroxidase from lettuce stems *African Journal of Biotechnology* (11), 2752-2756

[13] Rodgers, A. and Gibson, Y. (2009) Enzyme Kinetics. In: Plant Metabolic Networks, Ch. 4, Springer

[14] Khanna, V. K. (2008). New-generation nano-engineered biosensors, enabling nanotechnologies and nanomaterials. *Sensor Review,* 28 (1),39-45.

[15]Chen, L.C., Chung, Y.C. and Chang, C.T. Characterisation of an Acidic Peroxidase from Papaya (*Carica papaya L.* cv Tainung No. 2) Latex and its Application in the Determination of Micromolar Hydrogen Peroxide in Milk. (2012). *Food Chemistry*, 135, 2529–2535.

[16]Aghelan, Z. and Shariat, S.Z. (2015). Partial Purification and Biochemical Characterization of Peroxidase

from Rosemary (*Rosmarinus officinalis L.*) Leaves. Advanced *Biomedical Research* 4, 159.

[17] Deepa, S.S. and Arumughan, C. Purification and Characterization of Soluble Peroxidase from Oil Palm (Elaeis guineensis Jacq) Leaf (2002). *Phytochemistry*, 61, 503–511.

[18] Lakshmi, S., Shashidhara, G.M., Madhu, G.M., Rhea, M., Vivek, H.K. and Prasad M.N, (2018). Characterization of peroxidase enzyme and detoxification of phenols using peroxidase enzyme obtained from *Zea mays L* waste · *Applied Water Science* 8,207

[19] Vernwal S.K., Yadav, R.S.S. and Yadav, K.D.S (2006), Purification of a peroxidase from *S. melongena* fruit juice, *Indian Journal of Biochemistry and Biophysics* 43, 239-243.

[20] McEldoon, J.P., Pokora, A.R., and Dordick, J.S. 1995. Lignin peroxidase-type activity of soybean peroxidase. *Enzyme Microb. Technol.* 17 359–365

[21] Ankudey, G.E and woodey, M.Y. (2014) Assessment and Characterization of Peroxidase Activity in Locally in Grown Bean varieties from Ghana Grown Bean varieties from Ghana Grown Bean varieties from Ghana. *International Journal of Scientific and Research Publications,* 4, (6), 1-5

[22] Ruckminee, M, Gaurav, N. and Mishra, S.K (2012). Comparative Study on Peroxidase Activity in Different Plant Leaves. *Int. J. Curr. Sci. Tech.*, 1, 1,

[23] Jouili,H., Houda, B., Michel, R., Borderies, B and Jamet, E. et al..(2008). Partial purification and characterization of a copper-induced anionic peroxidase of sunflower roots. *Plant Physiology and Biochemistry*, Elsevier, 2008, 46 (8-9), 760-7.

[24] Braham, Y., Barhoumi, H. and Maaref, A. (2013). Urease capacitive biosensors using functionalized magnetic nanoparticles for atrazine pesticide detection in environmental samples.*Anal. Methods*, 5, 4898-4904.

[25] Hipolito-Moreno, A., Leon-Gonzalez, M.E., Perez-Arribas, L.V. and Polo-Diez, L.M. (1998). Non-aqueous flow-injection determination of atrazine by inhibition of immobilized tyrosinase. *Anal. Chim. Acta.* 362, 187–192

[26] World Health Organisation. Guidelines for Drinking Water Quality. World Health Organization; Geneva, Switzerland: 2008.