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EFFECT OF DIFFERENT HEAVY METALS ON α-AMYLASE ACTIVITY ISOLATED FROM COWPEA (*Vigna unguiculata* (L.) *WALP*.) GROWN ON CONTAMINATED SOIL FROM KACHIA LOCAL GOVERNMENT AREA OF KADUNA STATE, NIGERIA

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ABSTRACT

Recently, heavy metals reaching food crops is becoming the world concerns and their effect on humans. To avoid this, plants are used to reduce certain contamination of heavy metals. In this paper, heavy metal concentrations and alpha-amylase activity in plants grown on the contaminated soil was investigated. The objectives of the study were; to evaluate heavy metal concentrations in cowpea plant grown on contaminated soils, to extract and characterize Alpha amylase from the plant. Soil and cowpea plant samples obtained from polluted and unpolluted (control) area, as well as cowpea seed variety samples (7) obtained from IAR ABU Zaria, were digested and analyzed using Atomic Absorption Spectrometer (AAS). The alpha-amylase activity was also determined using a conventional method. Correlation analysis was also done among heavy metals in the soil and the leaves. Michaelis constants (Km) and maximal rates of substrate hydrolysis (Vmax) were determined Using Lineweaver-Burke plot. The plants grown on contaminated soil showed synergistic effect on the removal of Pb 109 mg/ml and Fe 49.5 mg/ml. The result of the correlation analysis for Cu, Pb and Fe showed positive relationships among these heavy metals in both contaminated and uncontaminated soils while Zn showed a negative correlation. The activity of the enzyme 30.0 µmole/min was found to be higher in seeds when compared with other samples. The enzyme optimum pH showed mixed activity due to iso-enzymes. The optimum temperature 60^0C produced activity of 10 µmole/min. The results imply that, plants growing in the vicinity of land used for military activities may pose some negative effect on the alpha-amylase activity and may affect the food chain.

Keywords: Heavy Metal; Removal; Alpha-Amylase; Cowpea; Military Activities

INTRODUCTION

α-Amylase is an enzyme that is found in plants, animals, and microorganisms and it has been used for over centuries in medicine, textiles, fermentation, and food industry [1] *α*-Amylases catalyzes the breakdown of starch into dextrin then gradually into reduced polymers of glucose molecules by acting on α-1,4-glycosidic bonds which can be used in the industries and for general application of biotechnology [2,3]. It is an important hydrolase that plays a significant role in the anaerobic digestion process which can increase the rate of breakdown of large sludge particles in other to uphold the competence of wastewater treatment and also provide more surface area for microbes to attach [4,5].

Some metal salts are the key to amylase properties because the binding of free electrons particles to enzymes increasing its activity andsteadiness. For instance, the role of Ca^{2+} on the alpha-amylasehas been confirmed [6,7]. Ca^{2+} and in addition with manganese have a beneficial outcome to alpha-amylase extricated from *Bacillus* shown to increase the activity while other divalent metals (Fe^{2+}, Cu^{2+} and Zn^{2+}) emphatically hinder enzymatic action [3]. A large portion of the α-amylase is selected as a mock-up of hydrolase in sludge for investigating the impact of Cu^{2+} and interaction mechanism amongst Cu^{2+} and α-amylase on sludge hydrolysis.

The military ground is a region utilized for a particular reason. Different sorts of army bases, ammo and explosives are utilized in such regions. In this manner, the soil in the military ground is often contaminated with heavy metals in view of consistent pollution on the soil (chemical influence, physical impact, thermal impact), vegetation differs alarm, the harsh environment is formed [8]. During the long year of military grounds exploitation, cartridge shells continually get into the soil. Usually, a cartridge of ammunition comprises of a copper-covered shell and a lead core. The impact of ammunition leads to high accumulation of lead and copper in soils of military grounds [9]**.** The past specialists were just centered around one angle, for example, toxin focus appropriation or biological hazard, while this investigation put accentuation on different variables, which incorporate contamination assessment.

Heavy metal, a global concern finds their way into the environment, food crops, water bodies due to inability to degrade easily leading to bioconcentration and bioaccumulation. The main source of heavy metal pollution is from the natural and human activities [10], that includes industrial, mining, urbanization, agricultural activities and the extensive applications of metal-based nanoparticles (MNPs) [11]. Previousreview also reported that, heavy metals in soil are taken up by rhizosphere of plants and even transported, promoted high accumulation in food crops [12]. Currently, the progressive increase in anthropogenic activities further enhancing the environmental absorptions of heavy metals in the soil [11]. Hence, it is required to explore more on the interaction between plants and heavy metal in the rhizospheric environment because the rhizosphere, not only is a vital route in the transfer of heavy metals in plant, still as well plays an important role in preventing heavy metal from reaching the agricultural products [13]. Most of the anthropogenic activities cause the accumulation of heavy metal leading to stress condition such as biochemical and development activities of the soil microorganism by undergoing several alterations, changes in soil composition, dissemination of chemical component (Fig. 1). There are various reports on the reduction of microbial diversity and activities in soil [14,15]. Different activities of microbial communities are very important in the soil, they act to bring about different and metabolic processes to decomposed organic pollutants and produce essential compound [16]. Effect of heavy metal to plants and microbes depends on the physicochemical property of the soil, the concentration of pollutant involved, soil acidity, plant species, thecontent of organic matter in the soil, clays materials. Wyszkowska and Kucharski [17] reported heavy metal effects on soil rhizomes.

The study was aimed to investigate the effect of heavy metals on alpha-amylase activity in cowpea grown on contaminated soil.

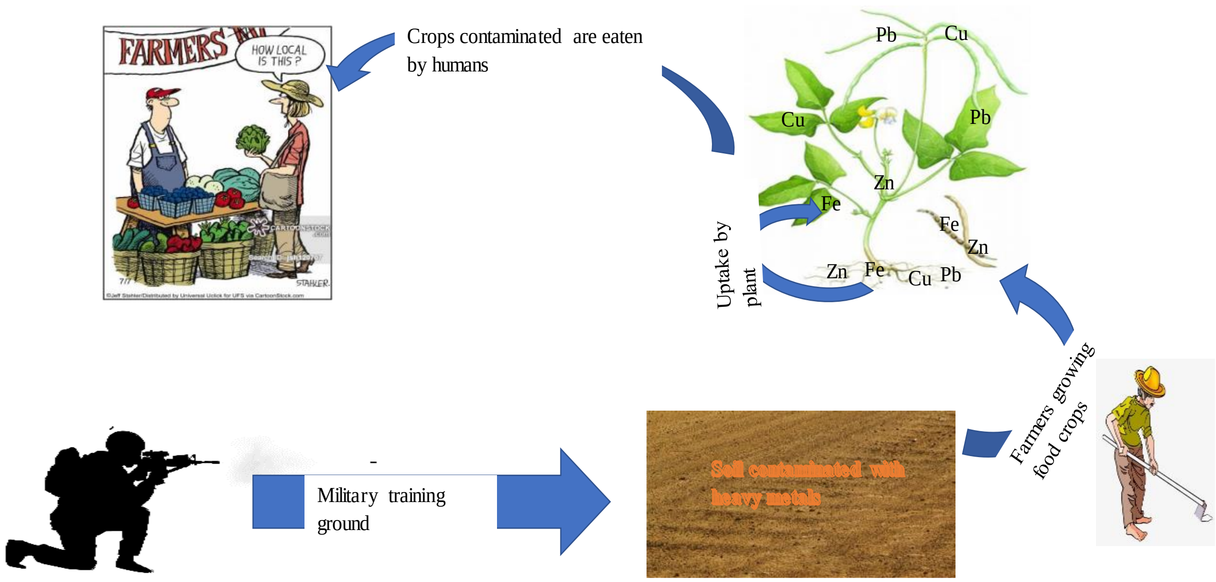


Figure 1: The lifecycle of heavy metals from military territories

MATERIALS AND METHODS

Sample Collection

The soil samples (1 and 2) were collected randomly from different locations in Kachia town where the military used for war games, at a depth between 0-15 cm using hand auger and plastic trowel. Another soil sample was collected from non-contaminated farmland at Sabon Gari area at a depth between 0-15 cm using hand auger and plastic trowel to be used as a control. The soil samples were air-dried and put into polythene bags and stored in a dry place for subsequent analysis and planting of cowpea.

Cowpea seed (variety; SAMPEA 7) that is least susceptible to contamination as certified by the seed production unit of the Institute for Agricultural Research (IAR), Ahmadu Bello University Zaria (ABU, Zaria) was purchased. The seed was then taken to the Laboratory of Biochemistry Department, ABU, Zaria, Nigeria where it was picked, cleaned of all debris and broken seeds. The seeds were later stored in a plastic container at room temperature (27-30^{o}C) for subsequent analysis and planting.

Cowpea (*Vigna unguiculata* (L.) Walp., the seed was grown in pots in uncontaminated soil (control) and in the contaminated soils with heavy metals stress. Each pot contained 3 kg of air-dried soil. The grown plant samples were collected on the 30th day. After planting, the metal analysis and characterization of the alpha-amylase enzyme were carried out immediately after harvesting the fresh plants.

DTPA-TEA Extraction Method

The collected soil samples were crushed in a clean ceramic mortar using a small ceramic pestle. The crushed soils were passed through 2-mm sieve to get a fine soil fraction. The fine soil fraction was used to extract heavy metals using the Diethylenetriaminepentaacetic Acid (DTPA). A 10 g of soil samples was mixed with 20 ml DTPA (0.05 M adjusted to pH 7.3 with TEA), then shaken on a reciprocation shaker for 30-45 minutes before filtering through Whatman No.1 filter paper. The filtrate was analyzed for heavy metals (Cu, Pb, Zn, Fe,) determination with Atomic Absorption Spectrophotometer (AAS), Perkin Elmer model 500.

Hydrochloric Acid Extraction Method

For the cowpea plant, the harvested leaves having been air-dried was placed in a forced-draft oven at 550℃ for about 12 hours, then grounded and passed through a 0.5 mm sieve. A 0.50 g sample was heated to ash form in a porcelain crucible for 4-6 hours at 500℃. The residue (ash) was dissolved in 25 ml of 1 M hydrochloric acid. Analysis for Fe, Cu, Zn, Pb, was done on the ash solution using Atomic Absorption Spectrophotometer [18].

Crude Extraction of Enzyme

Two grammes (2 g) of leaves and seed were homogenized using mortar and pestle in 0.05 M sodium phosphate buffer pH 7.0 (5 ml per gram fresh weight). The extract was filtered and centrifuged for 15 min at 4°C at 8000 rpm. The supernatant was collected which contained the enzyme and stored at 4°C [19].

Assay of Alpha-Amylase
The alpha-amylase activity was determined using 3, 5-dinitro salicylic acid reagent (Method 1) [19].

Effect of pH on the Activity of Extracted Enzyme (Alpha-Amylase)
Alpha-Amylases activities were estimated using 3 ml of phosphate buffers each of 0.05M having pH range from 2.0, 3.0, 4.0, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 9.0,10.0

Effect of Temperature on the Activity of Extracted Enzyme (Alpha-Amylase)
The optimum temperature for the activity of alpha-amylase was determined by incubating the mixture of 0.5 ml enzyme solution and 0.5 ml starch solution in 0.02 M phosphate buffer (pH 6.5) at 20, 40, 60, 80, 100^{O}C for about 10min.

Kinetic Analysis
Kinetic constants were determined by varying the starch concentration from 0.002 to 0.01 mg/ml and assaying α-amylase activity. Vmax and Km values were calculated using the Lineweaver- Burke plot.

Effect of Divalent Ions
Salts of Chloride; $CaCl_2$, $MgCl_2$ and $ZnCl_2$, were used each at a concentration level of 1 mMto determine their effects on the activity of alpha-amylase. To 0.5 ml of enzyme solution, 1ml of the respective salt solution was added and incubated for 1h in 0.3 ml sodium phosphate buffer (pH 6.0). The activity of the enzymes was assayed without any metal and was considered as control. The activity was taken as 100% and was done in triplicate.

RESULTS AND DISCUSSION

The Concentrationof Heavy Metal in Soils
The study aimed to investigate the heavy metal effect on alpha-amylase activity on cowpea grown on contaminated soil. From the results, both soils from the military area were contaminated with heavy metals as compared to the control soil. The concenrations of heavy metals were 333.04 mg/ml Fe, 44.31 mg/ml Pb, 46.73 mg/ml Zn and 63.16 mg/ml Cu. Heavy metals content in contaminated soil were quite high (Table 1). The source of soil contamination by heavy metals may be as a result of highly military effluents on the polluted area which was collected from Kachia, Kaduna State. The variation of heavy metals concentrations in the soil in different samples was significant ($P \leq 0.05$).

The result indicated that concentration of lead and iron were significantly ($P \leq 0.05$) higher in sample 2 and 1 respectively when compared with the control. Lead 368.82 mg/ml in sample 2 was significantly higher when compared with 44.31mg/ml sample 1and also iron content in sample 1 were significantly ($P \leq 0.05$) higher than sample 2 having values 333.04mg/ml and 94.26 mg/ml respectively. Zinc and copper presented much less concentration in all samples. For control, iron and zinc concentrations were significantly ($P \leq 0.05$) higher with values 71.92 mg/ml and 38.36 mg/ml.

Table 1: Concentration (mg/l) of heavy metals in contaminated soils and control

Metals					
Location	**Sample**	**Cu**	**Zn**	**Fe**	**Pb**
LOC1 (0m)	Soil 1	63.16 ± 0.81^{a}	46.73 ± 013^{d}	333.04 ± 0.27^{a}	44.31 ± 0.11^{d}
LOC2 (200m)	Soil 2	7.82 ± 0.64^{b}	9.81 ± 0.30^{e}	94.26 ± 0.8^{b}	368.82 ± 0.15^{e}
Sabon Gari	Control	2.96 ± 0.31^{c}	38.36 ± 001^{f}	71.92 ± 0.06^{c}	15.88 ± 0.21^{f}

Data are expressed as mean ±SD of triplicate measurement

Mean with different superscript along column differ significantly ($P \leq 0.05$)

Table 2: Concentration (mg/ml) of heavy metals in leaves of cowpea planted on contaminated soil and control

Metals					
Location	**Sample**	**Cu**	**Zn**	**Fe**	**Pb**
LOC 1(0m)	Soil 1	20.1 ± 0.01^{e}	4.1 ± 0.21^{b}	49.5 ± 0.18^{d}	11.4 ± 0.33^{a}
LOC 2(200m)	Soil 2	3.5 ± 0.12^{d}	5.2 ± 0.34^{c}	27.4 ± 0.43^{e}	109.0 ± 0.09^{b}
Sabon Gari, Zaria	Control	1.4 ± 0.35^{f}	3.2 ± 0.10^{a}	21.1 ± 0.67^{f}	6.1 ± 0.23^{c}

Data are expressed as mean ±SD of triplicate measurement
Mean with different superscript along column differ significantly ($P \leq 0.05$)

The Concentrationof Bioavailable Heavy Metals in Leaves

Table 2. Cu and Zn presented much less concentration in sample 2 (leaves) than lead and iron with values 20.1 mg/ml, 5.2 mg/ml, 109 mg/ml and 27 mg/ml respectively. The concentration of Cu and Fe (leaves) were significantly ($P \leq 0.05$) higher when compared to Zn and Pb.

Lead content was relatively high in leaves of cowpea in the entire samples when compared with the other heavy metal. Lead enters the body system through drinking water, food crops and air that accumulates in the body causing different cell damagedand can only be removed by remediation [20,21]. The high levels of Pb in cowpea leaves may probably be attributed to military activities (Shooting exercise) and pollutants in irrigation water. The level of Pb reported in this study is higher compared to those reported for orange, apple, banana and watermelon as 0.15 mg/ml, 0.76 mg/ml,0.02 mg/ml and 0.30 mg/ml respectively as reported by [22,23]. There was an uptake of all the metals from soil to plant in the military area as well as a control site. This is in agreement with the report of Akoto *et al.* [24]. The bioavailable concentrations of heavy metals were less in leaves samples when compared with soil samples. According to Demirezen and Aksoy [25], the content of heavy metals in leaves used in the studywas low. It may be due to root activity, which acts as a barrier for heavy metal uptake [26]. Properties of the soil determine the rate of metal absorption such as organic matter, microbial community, and pH and this indicated that, concentration of heavy metals in soil is not always comparable with concentrations in plants.

Heavy metal mobility and toxicity depend on the plant and heavy metal species [27].In this study, the concentration of Copper was as high as (20.1 mg\ml) in cowpea leaves from the site "1". Besides, the results obtained were found to be higher compared to the findings of Kamal *et al.*[28]on *Ipomoea aquatica* and *Enhydra flactuans*. Although, copper found in the soil is as a result of its natural existence and due to the higher mobility of Cu [29], and low retention than other toxic cations [30]. This study agrees with the results showing Pb and Fe accumulation in the soil is moderately lower than that of Cu in plants. However, plants need Cu for their growth which is usually ensured through artificial or organic fertilizers [31]. High copper concentration in foods causes metal fever in man and animal after bioaccumulation and also, inhalation of dust and vapors of copper can irritate the nose, mouth, and eyes and cause headaches, dizziness, and diarrhoea [32].

Transfer Factor

The mobility of heavy metal in the leaves studied in terms of abundances was in order: Pb >Fe>Cu>Zn. The Transfer factor values 0.448, 0.318, and 0.473 were found for Cu in sample 1, 2 and control, respectively which is significantly higher when compared with Pb and Fe (Table 3).

Transfer factor shows the effectiveness of the plant to transfer heavy metals from roots to their aboveground biomass [33]. Through effective root uptake and root-to-shoot transportation, heavy metals are uptake from the soil contaminated by collecting the above ground components [34]. Plants are known to take up and accumulate trace metals from contaminated soils; hence detection in plant leaves and crop samples was not surprising. Also, the variation in values obtained for these heavy metals in the soil and crop plant sample as against those from control sites is an indication of their mobility from the military area to the farmlands around particularly through leaching and runoffs. This also agreed with a report on the effects on copper and cadmium transfer from lettuce to snail [13].

Table 3: Transfer factor of Pb, Cu, Zn, and Fe for the soils to cowpea leaves

Samples	Cu	Zn	Fe	Pb
Soil 1	0.318 ± 0.23^{a}	0.088 ± 0.01^{d}	0.149 ± 0.01^{e}	0.257 ± 0.02^{e}
Soil 2	0.448 ± 0.03^{b}	0.530 ± 0.08^{a}	0.291 ± 0.05^{f}	0.296 ± 0.00^{c}
Control	0.473 ± 0.10^{c}	0.083 ± 0.00^{c}	0.293 ± 0.02^{g}	0.384 ± 0.20^{d}

The Correlation Among the Concentrations

Table4.showed the correlation among the concentrations of heavy metals in soils and cowpea leaves from the military area which shows that there was a strong correlation. Cu and Pb showed positive correlation and are significant. While Zn showed negative correlation and is not significant. However, Fe correlated positively and is not significant. Leaves accumulate the highest quantity of heavy metal in the order as follows: Sample 2 > 1 > Control. Tissues analysis of the 3 leaves growing in contaminated soil accumulated more heavy metals than those growing on uncontaminated soils.

This result corroborated the finding of Oves *et al.* [35]. that heavy metal in soil has significant interaction with the plants. This indicated that the cause of contamination is from the study zone and releases of heavy metals as a result of military activities are implicated. This agreed with Akoto *et al.* [24] who reported a correlation analysis of metal concentrations indicating Pb/Cu correlated. The correlation greater than 0.7shows a strong correlation[36]. Some of the heavy metals showed no significant correlation with each other, suggesting that metals may come into the soil from different types of wastes [28].

Table 4: Correlations between heavy metals contents in soils and in leaves of cowpea plant

	Cu leaves	**Fe leaves**	**Pb leaves**	**Zn leaves**	**Cu soil**	**Fe soil**	**Pb soil**	**Zn soil**
Cu leaves	1.000*							
Fe leaves	0.596ns	0.390ns						
Pb leaves	0.375ns	0.045ns	1.000ns					
Zn leaves	0.456ns	0.341*	0.366ns	0.775ns				
Cu soil	1.000*	0.596ns	0.376ns	0.412ns	0.599*			
Fe soil	0.587ns	0.490ns	0.052ns	0.790ns	0.596ns	0.390ns		
Pb soil	0.792*	0.085ns	0.966*	0.725ns	0.090ns	0.994ns	1.000ns	
Zn soil	0.801ns	0.829ns	0.606 ns	0.895ns	0.510ns	0.287ns	0.325ns	-

*-Correlation is significant and ns- Correlation is not significant at the $p \leq 0.05$ level (2 -tailed).

Characterization of Alpha-Amylase

Alpha-amylase was affected by the stress of heavy metals as compared in Fig. 2. The results show significant inhibition of enzymatic activities by a high level of soil contamination. The optimum pH for alpha-amylase activity was in the range of pH 4-7.5 that's for both the sample. The 3 Sample 1, C and A were found in the range of 40-80°C, with the rise in temperature the activity of alpha-amylase was increased with increase in temperature up to 80°C.

Heavy metal inhibits the enzyme activity by hindering their functional groups or binding with their substrate. Enzyme activities are influenced in different ways by different metals due to different chemical affinities of the enzymes in the soil system. The decrease in soil microbes and enzyme activities triggered by metal contamination negatively disturb soil fertility.

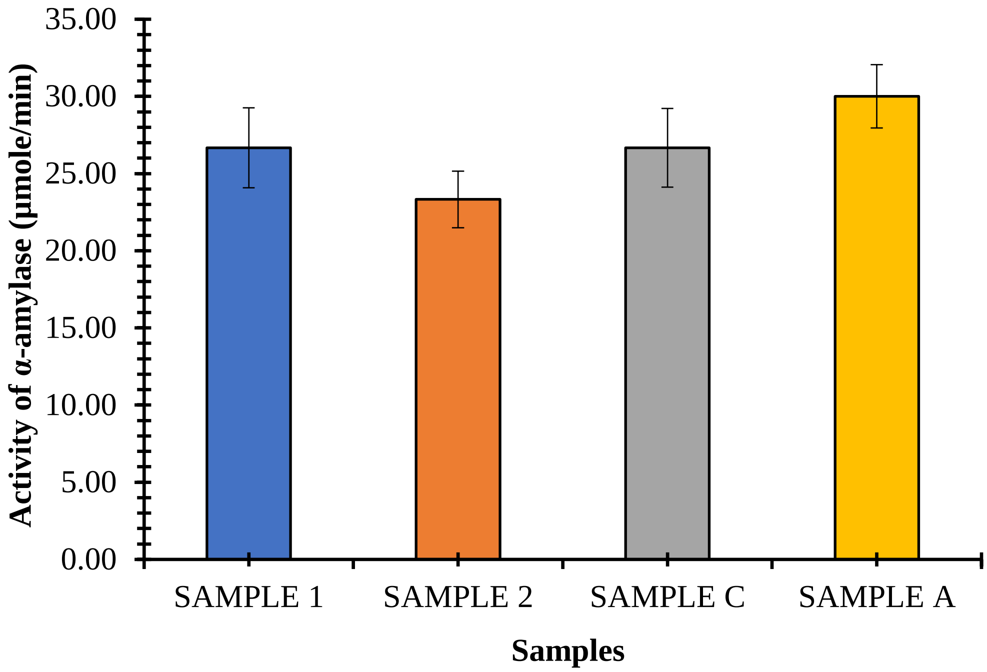


Figure 2: The activity of alpha-amylase in seed and cowpea leaves grown on the soils

Effect of pH on the Activity of Extracted Enzyme (Alpha-Amylase)

Graphfor pH outline thatthe α-amylase work over a wide range of pH (2.0–10.0). The optimum enzyme activity appeared at pH 7.0. Maximum activity was observed at pH 7.0 and extremely low at pH 4.0. The alpha-amylase activity was considerably decreased at low acidic as well as at high basic pHs because alpha-amylase is inactive in the acidic medium or due to the presence of heavy metals. Further increase in the pH above pH 8 result in a decrease in the activity of alpha-amylase because the enzyme retained most of its activity in the alkaline conditions (Fig. 3).

These results were comparable to earlier reports [38, 39] who reported that the optimal pH of different α-amylase of wheat has broad pH optimum range from 5.0 to 7.0. There was mixed activity due to the presence of isoenzymes because it is a crude sample which makes beta-amylase dominate the alpha-amylase in so many ways. The hydrolysis of starch by α-amylase is highly affected by pH. Kim *et al. s*[39]reported that alkaline amylases adopt a unique structure and catalytic mechanism to adequate function on the starch carbohydrate in these alkaline conditions. They also suggested that, the existence of an un-ionized carboxylic acid group in the active site of GM8901 alpha-amylase at high pH values [40].

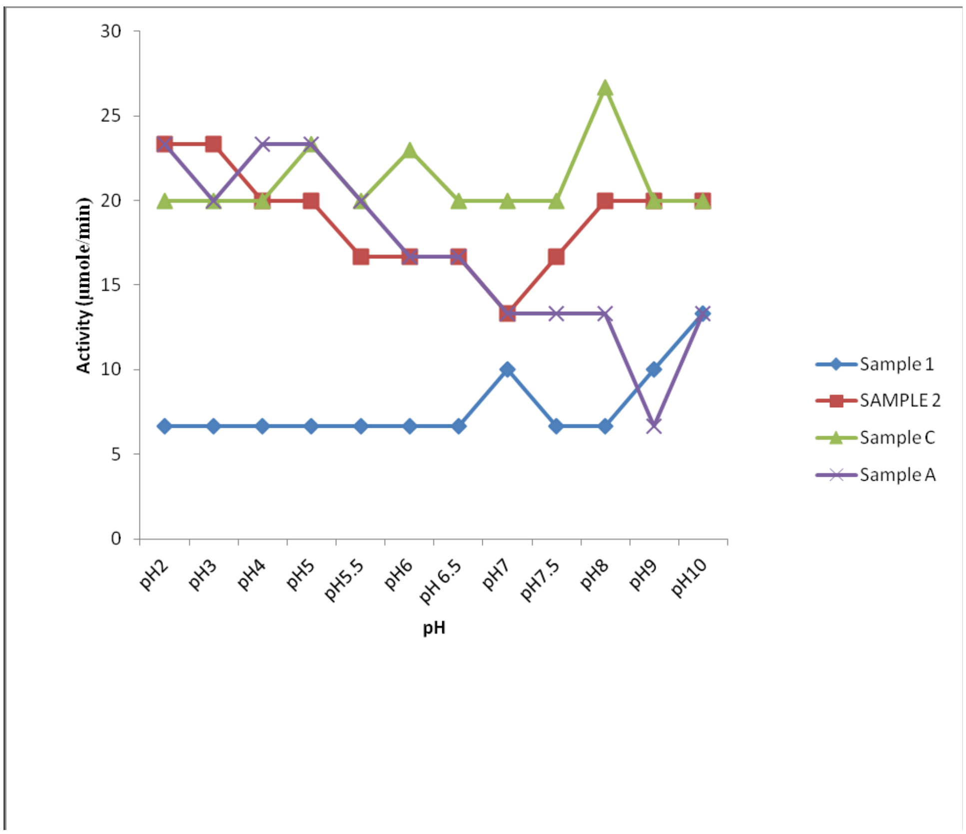


Figure 3: Effect of pH on alpha-amylase activity in cowpea seeds and leaves grown on the soils

Effect of Temperature on the Activity of Extracted Enzyme (Alpha-Amylase)

Results showed the optimum temperature for alpha-amylase, was active in the temperature at 60 $^{\circ}$C, while the activity reduced slightly at temperatures beyond and less than 60 $^{\circ}$C (Fig. 4). The maximum stability of the alpha-amylase was detected in 40- 80 $^{\circ}$C and decreased with a risingtemperature above 80 $^{\circ}$C in the presence or in the absence of heavy metals.

The decrease of enzymes activity was found with an increase in temperature above 80 °C indicating a loss in the active conformation of the enzyme. These results were agreed with previous reports[41].Oziengbe and Onilude [42] suggested that an increase in temperature increases theactivity of the enzymes, above the optimum temperature it destroyed the enzymes[40].

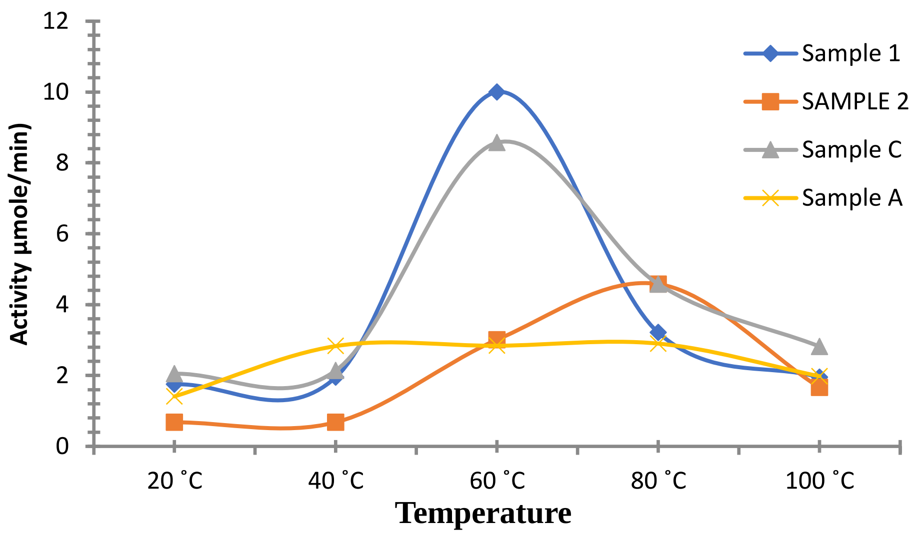


Figure 4: Effect of temperature on alpha-amylase activity in cowpea seeds and leaves grown on the soils

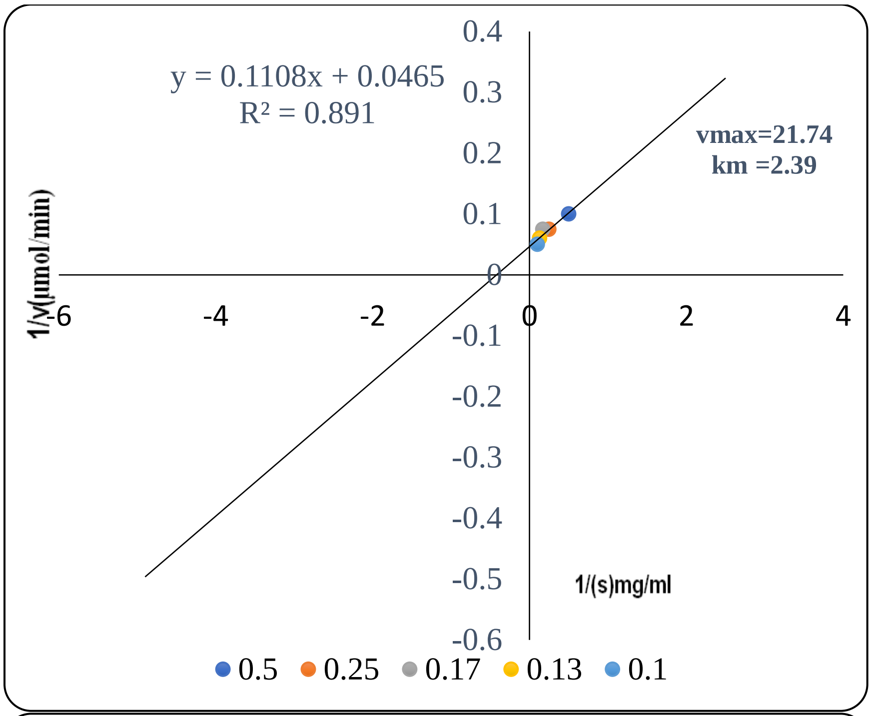
Kinetic Analysis

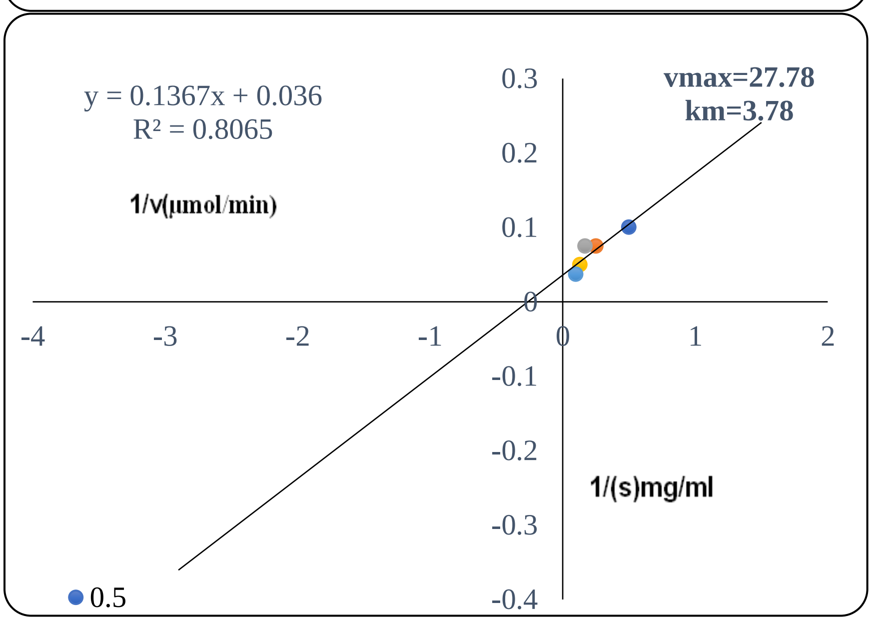
In the present study, Fig. 5 shows Lineweaver in the plot for the determination of kinetic parameters of the enzymes (Vmax and Km) by incubating the purified enzyme solution in the presence of different concentrations of soluble starch, 0.002–0.1 mg/mL. The Km and Vmax (Michaelis-Menten constant) of the seed, values were found to be 2.39 mg/ml and 21.74 µmol/min, respectively. The Km and Vmax from sample 1 and 2 were 3.78 mg/ml, 4.65 mg/ml and 27.78, 32.26 µmol/min respectively towards the soluble starch substrate. The Km and Vmax value for the control sample were 5.29 mg/mL and 23.81µmol/min, respectively for α-amylase. Km approximates the affinity of the enzyme for its substrate.

A small Km indicates high affinity, and a substrate with a smaller Km will approach Vmax more quickly. Table 5 shows the physiological efficiency Vmax/Km which explains that the highest Vmax/Km indicates strong affinity. In fact, in the study, the kinetic behavior of the crude enzyme from seed would be far excellent when compared to the other contaminated sample.

Goyal and co-researchers stated that Km and Vmax values for *Lactobacillus manihotivorans* to be 3.44 mg/ml and 0.45 mg hydrolyzed starch/ml/min at 55 °C, respectively [40]. From the results, the highest Vmax calculated for alpha-amylase hydrolyzed soluble starch was32.26 (µmol/min).

The Vmax value of α-amylase was lesser than the value reported by [43]. The Km of alpha-amylase was 4.65 mg/mL, which was higher and lower than that of BH072 α-amylase (4.27 ± 0.21 mg/mL) and McAA (6.40 mg/mL) [43]. This suggests that α-amylase has a moderately higher affinity for soluble starch.





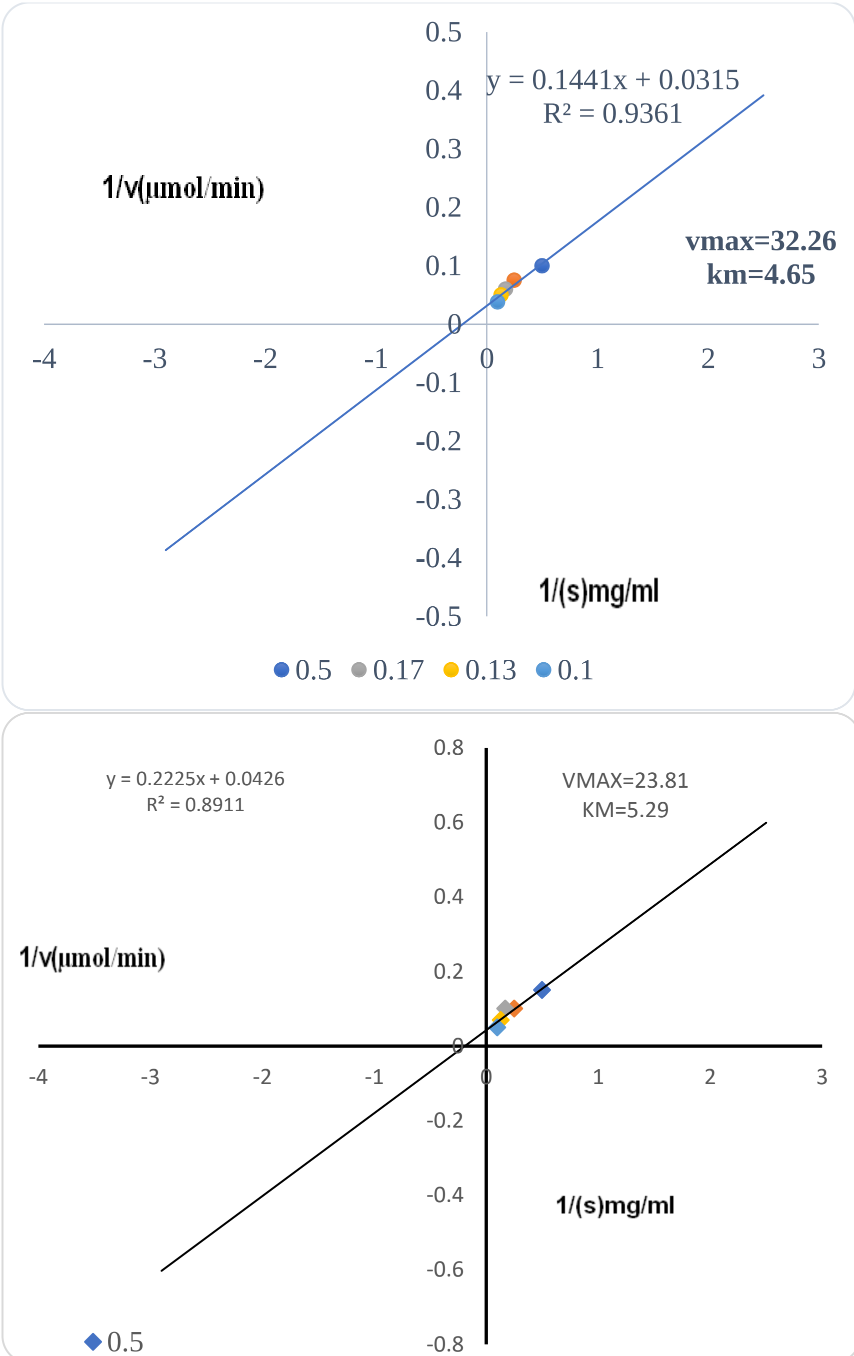


Figure 5: Lineweaver-Burke plot for the activity of alpha-amylase

Table 5: Kinetic parameters of alpha-amylase from cowpea seed and leaves grown on soil samples

Samples	Km(mg/ml)	Vmax (μmol/min)	Vmax/Km
Sample A	2.39	21.74	9.10
Sample C	5.29	23.81	4.50
Sample 1	3.78	27.78	7.35
Sample 2	4.65	32.26	6.94

Effect of Divalent Ions

The effect of metal ions ($Ca^{2+,}$ $Zn^{2+,}$ $Mg^{2+)}$ on the alpha-amylase activity was studied at a concentration of 1 mM. The enzyme activity was significantly increased by the presence of Mg^{2+} (Fig. 7).

External factors such as cation and additives have been known to affect the activity of the enzyme. Many trace elements are needed by different microorganisms for their growth as well as for many enzyme-catalyzed reactions. Ca^{2+} is found in alpha-amylase which is very essential to maintain the structural integrity of the active site. The hydrolytic activity of the crude enzyme was highly stimulated by $MgCl_2$, $CaCl_2$ and $ZnCl_2$ ions. In agreement with our results, the α-amylase was significantly increased by the presence of Zn^{2+} and Mg^{2+}[43]. However, enzymes are generally inactivated by heavy metals, with metal ions such as Cu^{2+} noted to cause irreversible inhibition of the enzyme by binding strongly to their amino acid backbone[41,44]. Machius and co-researchers reported that calcium ion improves the thermal stability by the creation of calcium–sodium-calcium in the main Ca^{2+} binding site, bridging the domains A and B of the enzyme [40,45].
Thus, the activities of the soil enzyme especially those of the soil amylase could be used as sensitive biological indicators of heavy metals pollution. The pH of the soil in Kachia was found to have played an important role in enhancing the toxicity of the heavy metals. The present work showed that amylase was not active in the presence of heavy metals. This could be an added advantage, as the sensitivity of amylase to heavy metal ions poses problems in some areas where military uses for their training due to the metal composition of reactors or the presence of metal.

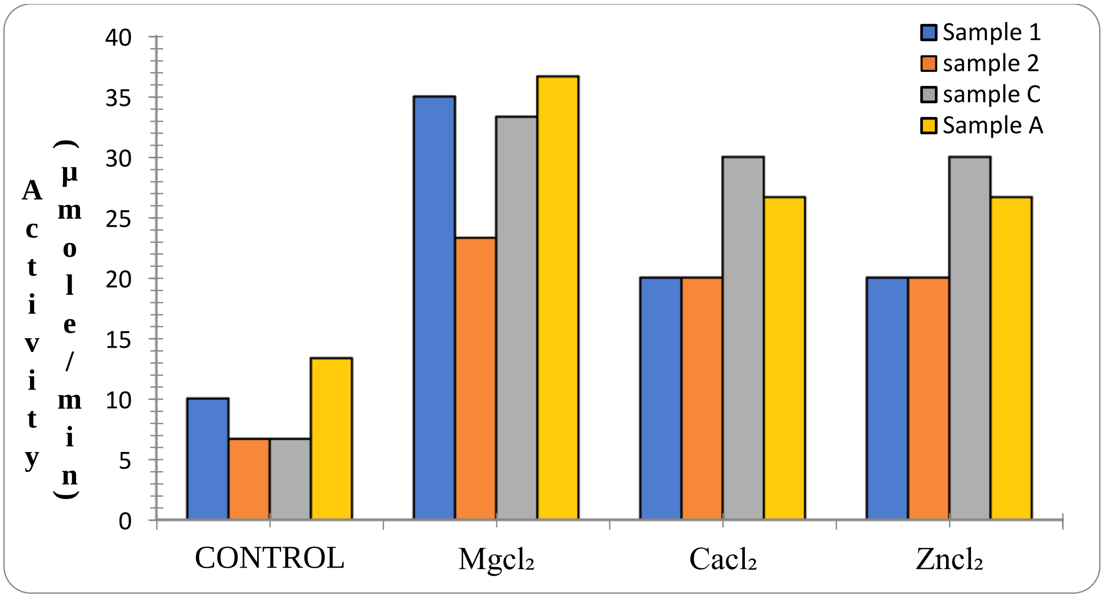


Figure 7: Effect of divalent cation on alpha-amylase activity in cowpea seeds and cowpea leaves grown on the soils

CONCLUSION

Investigation of the effects of heavy metals pollution on the soil enzymatic activities in Kachia area showed that the heavy metal contents of the soil in the area were exceeding the normal range. This is indicated by their strong antagonistic effect on the soil enzymatic activities. This study suggests a quick approach like bioremediation, should be put into practice to ensure effective organization of management strategy.

The military should consider the replacement of lead shot by non-toxic ammunition for their training. While these metals are in many ways' indispensable; good precautions and adequate occupational hygiene should be taken in handling them.

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