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Original article

Bioefficiency of *Clarias gariepinus* Liver Rhodanese and Physicochemical Parameters of Soil from Cassava Effluent Contaminated Odo-Oba River, Ogbomosho, Oyo State, Nigeria

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Abstract

Cyanide contamination of Soil and aquatic environment has become a great concern in Nigeria because of increase in the number of cassava processing milling plants. The sample collection and the conduct of this experiment took place between September and October when the country is experiencing late rainfall. The levels of physicochemical parameters in soil and plant samples were obtained using Atomic Absorption Spectrophotometer. Soil and plant samples of A*maranthus spinosus* were obtained from cassava processing site (site X) and other samples 100m to the cassava mills (site Y) as control. The concentration of metals in soil samples in mg/kg at site 'X' recorded were Cr(39.0mg/kg), Mn(3.5mg/kg), Cu(33.0mg/kg), Fe(4.3mg/kg), Pb(2.5mg/kg), and Zn(52.5mg/kg) while that of site 'Y' were Cr(16.5mg/kg), Mn(1.3mg/kg), Cu(13.0mg/kg), Fe(1.9mg/kg), Pb(0.3mg/kg), and Zn(17.0mg/kg). The concentration values for AS, Cd and Nickel in the environment were below detection limit for all soil and plant samples. The concentrations of metals in *A. spinosus* root and shoot in mg/kg from sites 'X' were Cr(6.00mg/kg - 8.50mg/kg), Fe(0.55mg/kg - 0.80mg/kg), Cu(4.00mg/kg - 5.50mg/kg), Pb(0.05mg/kg - 0.08mg/kg), Zn(6.00mg/kg - 8.00mg/kg), Mn(0.35mg/kg - 0.5mg/kg) while that of site 'Y' were Cr(5.50mg/kg - 8.00mg/kg), Fe(0.545mg/kg - 1.00mg/kg), Cu(4.00mg/kg - 6.50mg/kg), Pb(0.03mg/kg - 0.08mg/kg), Zn(7.50mg/kg - 9.50mg/kg), Mn(0.30mg/kg - 0.60mg/kg). The values of some metals analyzed were above the recommended values by WHO and FEPA. The analysis of effluents and surface water samples resulted in higher figures for most of the parameters and acidic pH in cassava effluent than the surface water sample. The mode of phytoremediation was also investigated. Data obtained suggested the plants could be used for phyto-extraction of these metals. Rhodanese, enzyme that detoxify cyanide was extracted and characterized from the liver of *Clarias gariepinus* of cassava effluents contaminated Odo-Oba River. The results show specific activities of 0.0526RUmg-1. The optimal temperature and pH of 30°C and 7.0 were recorded for the enzyme respectively. The Vmax of 9.62RU/Ml and 6.33RU/Ml were obtained for the KCN and $Na_2S_2O_3$ substrates while for the Km, higher figures of 49.4mM and 28.5mM were recorded respectively. However, the Km values of the fish liver Rhodanese of *Clarias gariepinus* indicated higher affinity for thiosulphate ($Na_2S_2O_3$) than for potassium cyanide (KCN) as a substrate, although maximum activity was observed for KCN substrate. Inhibition studies on the enzyme with a number of chloride salts showed that the activity of the enzyme was not affected by Mg^{2+}, Mn^{2+},Ca^{2+} while Ba^{2+},Hg^{2+} and Cu^{2+} inhibited the enzyme considerably.

Keywords: Bioremediation, Phytoremediation, Rhodanese, cyanide, thiocyanate.

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INTRODUCTION

Cyanide poisoning in the environment is a major challenge in the processing of cassava. The various stages of processing involve in the release of noxious substances to the immediate environment (Fred *et al*., 2014). Cassava is well known for the presence of free and bound linamarin and lotaustralin, and is converted to HCN in the presence of linamarase (O'Hair, 1995). Processing of cassava generates waste effluents which are deleterious and harmful to the environment (Obueh and Odesiri, 2016). Effluents from cassava processing if discharge into bodies of water cause death of aquatic lives due to oxygen depletion as a result of bacteria growth (Obueh and Odesiri, 2016). Aquatic organisms react differently to external contaminations (Amiya *et al*., 2012). As fishes ingest food and water, heavy metals is also ingested along thus making them an essential bioindicator of contaminants in an ecosystem. Aquatic organisms have been known to bioaccummulate trace metals which remain in their body for a considerable amount of time. Fishes have been reported to be good accumulator of organic and inorganic contaminants as well (Ishaq *et al*., 2011). Rhodanese (EC 2.8.1.1) is a sulphur transferase that catalyses, in vitro, the formation of thiocyanate from cyanide and thiosulphate or other suitable sulphur donors. It has been found to be active in all organisms (Akinsiku *et al*., 2010). Studies have shown that it is present in the nucleus, mitochondrion and cytosol, with a high concentration in the mitochondrion (Akinsiku *et al*., 2010). The major function of rhodanese is cyanide detoxification, especially in mammals in which cyanide is converted to the less toxic thiocyanate and excreted through the kidney (Akinsiku *et al*., 2010). The aim of this study was to investigate the effects of cassava mill effluents on soil and water physicochemical properties, Phytoremediation potential of *Amaranthus spinosus* and to determine the bioefficiency of *Clarias gariepinus* liver rhodanese from cassava effluent contaminated Odo-Oba River, Ogbomosho. This was achieved by ascertaining the presence of heavy metals, minerals and cyanide contents in the environment (soil, water and plants) around the cassava effluent contaminated site and also by determining the enzyme activity of isolated partially purified Rhodanese obtained from the liver of *Clarias gariepanus* from contaminated Odo-Oba River water.

MATERIALS

This research was conducted at Odo-Oba, Ogbomosho, Oyo State, in southwest Nigeria (Its coordinates are 7°28'0" N and 4°7'60" E in Degrees Minutes Seconds). It is a small community in Oyo State and the major activity of the residents is processing of cassava into garri. Cassava effluents from a cassava mill close to the river was collected directly into sterile plastic bottle. Surface water from the river was obtained, also, soil around the contaminated and control soil sample 100m from the mill. The cat fishes, *Clarias gariepinus* were obtained from Odo-Oba river located in Ogbomosho, Oyo state, Nigeria and the liver was extracted into ice packs followed by further analysis.

Plant material and authentication

Amaranthus spinosus were collected at the cassava mill effluent contaminated environment in Odo-Oba, Oyo State, Nigeria. The plant was authenticated (UILH/001/593) at the Herbarium Unit of the Department of Plant Biology, University of Ilorin, Kwara State.

Chemicals and Reagents

Chemicals and reagents used were of analytical grades, this includes: chlorine, calcium, phenolphthalein indicator, propyl alcohol, HCL, methyl orange indicator, Ammonia(NH_3),Nitrate, Sodium Hydroxide, Potassium Hydroxide, Phenoldisulfuric acid reagent,H_2SO_4,Potassium Nitrate, Sulfanilic acid reagent, Clove's acid reagent, silver nitrate, NaCl, iron, magnesium, aluminium,$CACO_3$, EDTA, Calcium, Acetate buffer, Sodium bicarbonate solution, Acetic, Copper sulfate. β-mercaptoethanol, urea, Ethylene diamine tetraacetic acid (EDTA), ɛamino-n-caproicacid, sodium borate, boric acid and bovine serum albumin (BSA) were obtained from Sigma Aldrich Chemical Company USA (SACCU). Teflon homogenizer, Beckman Optima LE-80K ultracentrifuge.

Plate 1. Odo-Oba Cassava Processing Sites [A**,** B**,** C=Sun drying of cassava-mash, D=the cassava-grinding machine, E=the draining rack, F= Cassava peel dump site (solid waste), G=soil from control site, H=soil from cassava effluent site]

METHODS

Preparation of Samples

The protocol outlined by (Boulding, 1994) was used in the samples' preparation

Determination of Physicochemical Parameters

The procedure of (George *et al*., 2013) was adopted to determine the physiochemical parameters of the soil samples, effluents and surface water.

Analysis of Heavy Metals of Plant

The protocol outlined by (Arise *et al*., 2015) was used to analyse the heavy metals in the roots, leaves and stem of the plant.

Determination of Phytoremediation Quotient

Translocation

The Translocation Factor (TF) was calculated using the procedure described by APHA (1998) expressed as:

TF = $[METALS]_{shoot}$/ $[METALS]_{root}$

The Bioconcentration Factor (BCF) as described by (Yoon *et al*.,2006) expressed as:

BCF = $[METALS]_{root}$/ $[METALS]_{soil}$

The Biological Accumulation Coefficient (BAC) was obtained by adopting the procedure of (Khan and Uzair2013) expressed as:

BAC = $[METALS]_{shoot}$ / $[METALS]_{soil}$

Enzyme preparation

The enzyme preparation was carried out by method outlined by (Akinsiku *et al*., 2010)

Determination of kinetic parameters

The Km and Vmax of the enzyme were determined by method outlined by (Lee *et al*., 1995) the kinetic parameters were estimated from the double reciprocal plot (Lineweaver and Burk 1934)

Enzyme and protein assays

Rhodanese activity was measured routinely according to the method of (Lee *et al.*, 1995). While concentrations of Protein were determined by the outlined method of (Gornall *et al*., 1949) using bovine serum albumin as standard.

Effect of temperature and pH on the enzyme

The temperature and pH effect on enzyme activity was determined by the outlined protocol of (akinsiku *et al*., 2009) was used.

Effect of cations on enzyme activity

The method adopted by (Agboola and Okonji, 2004) was used.

Statistical Analyses

The data of the physico-chemical, heavy metal content of soil samples, roots and shoots of plant samples and various biochemical parameters were expressed as Mean of three replicates ± standard deviation (SD). The data were subjected to analysis of variance and Tukey's multiple range tests using GraphPad Prism version 6.0 (GraphPad Software, San Diego, CA, USA).Differences were considered ssignificant at $p < 0.05$.

RESULTS and DISCUSSION

The results of the analysis of the physicochemical parameters showed decrease in pH values of the polluted soil (pH4.7) and presence of cyanide (2.3 mg/kg) with corresponding increase in the other parameters as compared to the control(pH 6.52),with cyanide below detection limit (Table 1). These pH values are lower than the standard values 7-8.5 (NSDWQ, 2007) for soil. The pH value of the polluted soil indicated that the soil was acidic which suggests that the cassava effluents imparted acidity on the soil. The acidity could be due to the presence of high cyanide contents in the cassava effluents (Uzochukwu *et al*., 2001). Liquid wastes from cassava processing facilities are being considered as deleterious and should be prevented from reaching cultivated lands (Eze and Onyilide, 2015). The pH of the soil affects the presence of nutrients and the potentiality of noxious materials as well as the physical features of the soil (Osakwe, 2012). However, various plants grow between 6.4 - 7.0 pH and soil acidity is one of the principal factors affecting nutrient availability to plants (Hajek *et al.,* 1990). Thus, the pH estimates of the present site under review indicated high capacity for increase level of metals' availability in the soil; hence, this brings about a risk in the high level of absorption of heavy metals by plants. The soil organic matter was high in cassava polluted soil (17.08) compared with the control (13.45) while the total Nitrogen, range from 0.154-0.251%. The overall number of nitrogen recorded was likely due to mineralization of nitrogen as a result of humic presence and nitrogen is an essential elements required by plant for normal growth (Osakwe, 2012). However, the capability of breaking down cyanide universally throughout ecosystem has been reported and possesses enzymatic properties that can be in the form of hydrolytic, oxidative and transfer or substitution in nature (Okechi *et al*., 2012). Such breaking down may result in the build up of organic materials and high carbon content of the soil (Igbinosa, 2015). The concentrations of Cu in plant roots samples is 4.0mg/kg while that of shoots ranges between 5.5-6.5mg/kg all in the acceptable range of 10mg/kg WHO standards. The soil concentration of Cu **(Table 1)** ranged between 13.0-33.0mg/kg. The permissible limit recommended by WHO (1996) is 36 mg/kg, thus, the values are within the permissible limits. The concentration of plant samples for Fe includes 0.45-0.55mg/kg for the roots and 0.8-1.0mg/kg for the shoots and these values were acceptable range of FAO (1976). The soil concentrations of Fe for all the sites ranged between 1.9-4.3mg/kg which is within the permissible limits 4.5ppm (Neetika and Ashwani, 2013). Mn concentration in plant samples for all sites varied between 0.3-0.35mg/kg of roots and 0.5-0.6mg/kg of the shoots. While for the soil samples, the Mn concentration varied between 1.3-3.5mg/kg. The permissible limit by (Neetika and Ashwani, 2013) is 2mg/kg. This indicated a high value above the permissible limits of Mn for soil. Since the studied sites is along the major road which experience heavy traffic daily, the high levels of the heavy metals in both the control and polluted sites agrees with the findings of (Ogundele *et al*., 2015) where high concentration of metals in the studied sites was attributed to the increase of traffic and vehicular emissions.

Table 1. Physicochemical parameters of soil samples

parameter	Soil(control)	Polluted	*Permissible value of soil	Unit
PH in Water	6.5 ± 0.058^{a}	4.7 ± 0.100^{b}	-	
Cyanide (CN)	BDL	2.30 ± 0.115^{b}	-	mg/kg
Organic Matter	13.45 ± 0.015^{c}	17.08 ± 0.006^{b}	2.0	%Carbon
Total Nitrogen (N)	0.154 ± 0.002^{a}	0.251 ± 0.001^{a}	0.20	%
Mn	1.3 ± 0.000^{a}	3.5 ± 0.208^{c}	2.0	mg/kg
Zn	17.0 ± 0.311^{c}	52.5 ± 0.200^{a}	0.6	mg/kg
AS	BDL	BDL	5.0	mg/kg
Fe	1.9 ± 0.006^{a}	4.3 ± 0.252^{b}	4.5	mg/kg
Pb	0.3 ± 0.058^{a}	2.5 ± 0.100^{b}	0.05	mg/kg
Cd	BDL	BDL	0.01	mg/kg
Cr	16.5 ± 0.252^{a}	39.0 ± 0.404^{c}	0.03	mg/kg
Cu	13.0 ± 0.118^{a}	33.0 ± 0.361^{b}	0.2	mg/kg
Ni	BDL	BDL	0.10	mg/kg
Exchangeable Ca^{2+}	0.80 ± 0.053^{a}	1.40 ± 0.058^{b}	10-20	cmol/kg
ExchangeableMg^{2+}	1.20 ± 0.04^{a}	1.50 ± 0.015^{a}	3-8	cmol/kg
Exchangeable Na^{+}	0.08 ± 0.003^{b}	0.12 ± 0.002^{b}	0.7-1.2	cmol/kg
Exchangeable K^{+}	0.07 ± 0.002^{a}	0.20 ± 0.001^{a}	0.6-1.2	cmol/kg

Values are expressed as mean ± SD from triplicates determinations BDL = below detection limit. Means on the same horizontal row between samples with same superscripts are not significantly different ($p>0.05$).*Source: FEPA (1991), Neetika and Ashwani, 2013.

The result of heavy metal analysis showed increased levels of heavy metals in the cassava effluents contaminated soil. The results showed that the heavy metal concentrations were generally higher at the soil receiving the effluents than the control soil. The concentration values for AS, Cd and Nickel in the environment were below detection limit for all soil and plant samples. Concentration of Zn for plants samples (Table 2) for all sites showed range of values 6.0-7.5mg/kg for the roots and 8.0-9.5mg/kg for the shoots. The acceptable limit by WHO (1996) is 0.06mg/kg which implied the plants samples were above the acceptable value. The soil concentration of Cr for all sites ranged between 16.5-39.0mg/kg was found to be above the acceptable limits 0.03ppm by FEPA (1991).The level of lead in plants roots' samples ranged between 0.03-0.05mg/kg while for shoots samples were 0.08mg/kg. The recommended limits for Pb by WHO (1996) is 2mg/kg. Thus, Pb concentrations for plant samples were within the permissible limits as shown in **(Table 2)**.The level of Pb in soil for the control is 0.3mg/kg which is within the permissible limits of 0.05ppm set by FEPA (1991),while for the soil receiving effluent; Pb value is 2.5 and was found to be above the permissible limits.

Table 2. Heavy metals concentration (mg/kg) in roots and shoots of *Amaranthus spinosus* in both the contaminated and control sites

HEAVY METAL	**SITE (mg/k)**	**ROOT (mg/kg)**	**SHOOT (mg/kg)**	***Permissible value of plant(mg/kg)**
Mn	X	0.35 ± 0.012[a]	0.50±0.115[b]	2.0
	Y	0.30±0.006[a]	0.60±0.173[b]	
Zn	X	6.00±0.173[a]	8.00±0.208[a]	0.60
	Y	7.50±0.115[b]	9.50±0.058[b]	
AS	X	BDL	BDL	_
	Y	BDL	BDL	
Fe	X	0.55±0.006[a]	0.80±0.115[b]	20.0
	Y	0.45±0.017[a]	1.00±0.153[b]	
Pb	X	0.05±0.006[a]	0.08±0.006[b]	2.00
	Y	0.03±0.010[a]	0.08±0.000[b]	
Cd	X	BDL	BDL	0.02
	Y	BDL	BDL	
Cr	X	6.00±0.289[a]	8.50±0.100[a]	1.30
	Y	5.50±0.115[b]	8.00±0.115[b]	
Cu	X	4.00±0.100[a]	5.50±0.058[b]	3.0
	Y	4.00±0.100[a]	6.50±0.173[b]	
Ni	X	BDL	BDL	10.0
	Y	BDL	BDL	

Values are expressed as mean ± SD from triplicates determinationsBDL=below detection limit, A=Control, B=soil contaminated with cassava effluents. Means on the same vertical column between samples with same superscripts are not significantly different (p>0.05).*Source: WHO (1996).

The metal accumulation characteristics of *Amaranthus spinosus* for the contaminated and control sites were carried out to determine its ability to absorb concentration of heavy metals from the soil to the roots (BCF), soil to shoots (BAC) and likewise from the roots to the stems and leaves of the plants (TF). From (Table 3), TF > 1 were found for all the metals in both control plants and plants in contaminated cassava effluents. This suggested that these plants have capabilities to be used for phyto-extraction of these metals (Arise *et al*., 2015). The process of phyto-extraction involved the translocation of heavy metals to the shoot part of plants (Yoon *et al*., 2006). This indicated *A. spinosus* might be a good and efficient choice for extracting Pb, Fe and Mn followed by Cu, Cr and Zn. The BCF is the ability of *A. spinosus to* extract heavy metals from the soil. In this study, the BCF values < 1 for all the metals showed that *A. spinosus* might not be a good choice for phyto-stabilization as it tends to show intolerance to the heavy metals concentration in the soil. The BAC values as compared to the BCF values of corresponding metal concentrations showed the plants tends to have much accumulation in their shoots (edible part) from the soil, thereby increasing metal availability in food chain. These results clearly showed that consumption of this vegetable and grazing of animals should be greatly avoided.

Table 3. Phytoremediation determination of Amaranthus spinosus for Metals in the contaminated and control sites

HEAVY METAL	SITE	TF	BCF	BAC
Mn	X	1.43	0.27	0.39
	Y	2.00	0.09	0.17
Zn	X	1.33	0.35	0.47
	Y	1.27	0.14	0.18
Fe	X	1.46	0.29	0.42
	Y	2.22	0.11	0.23
Pb	X	1.60	0.17	0.27
	Y	2.67	0.01	0.03
Cr	X	1.42	0.30	0.52
	Y	1.46	0.14	0.21
Cu	X	1.38	0.00	0.31
	Y	1.63	0.20	0.20

X = control plants, Y=plants in cassava effluents contaminated soil.

TF = plant shoots to roots metal concentration ratio.

BCF = plant roots to soil metal concentration ratio.

BAC = shoots to soil metal concentration ratio.

Table 4 showed the physicochemical characteristics of cassava effluent collected from Odo-Oba local cassava mill in comparison to the surface water in the area, the health impacts of the high contents of the parameters and The Nigeria Standard for Drinking Water Quality (NSDWQ). From the results, there are higher values for most of the parameters such as for the heavy metals, CO_2 and Chloride in effluent than the water sample. While values for Hg, Ni, Cr, As and Cd were not determined. The values of Zn, Cu, NO_3, SO_4, F^- , Na and Cl^- were within the permissible limit of NSDWQ (2007) in both the cassava effluent and surface water. Values for Fe^{2+}, Mn^{2+}, Pb^{2+}, Turbidity, Al^{3+} and cyanide were above the permissible limit of NSDWQ. The cyanide content of the effluents is (1.17mg/l, pH 5.3). The characteristics of the effluent were in resemblance to the work done by (Omomowo *et al*., 2015) and this property is necessary for toxicity and antimicrobial activity (Okunade and Adekalu, 2013). These findings necessitate for sufficient handling and provision for wastewater discharge into the environment. Likewise the values for magnesium and sodium hardness, magnesium and sodium Total were higher in the cassava effluent than the surface water. However, the similarities in the physicochemical characteristics indicated high contamination from the processing plant to the surface water. The value of TDS ranged between 218-362mg/l in the acceptable range of less than 500 mg/l WHO (2004). Conductivity ranged between 89.04 – 103.57 µS/cm. The odour of the cassava effluent was repulsive which might be due to high level of hydrogen sulphide (1.3mg/l). The permissible limit of hydrogen sulphide by NSDWQ (2007) is 0.05mg/l. BOD level ranged between 0.4-3.6mg/l and American Society of Civil Engineers (ASCE) standard of greater than 4mg/ml as a threat to life (Okunade and Adekalu, 2013). The COD Values ranged between 0.6-5.8mg/l. The BOD and COD values (6.3mg/l, 10.4mg/l) from the surface water sample are above the WHO (2004) guidelines for drinking water (6 mg/l, 10 mg/l). While The BOD and COD values (7.4mg/l,11.6mg/l) from the cassava effluent sample exceeded the WHO (2004) guidelines of 4 mg/l. This indicated that the effluent is hazardous and deleterious to the organisms in the area and the environment at large.

Table 4. Physicochemical Parameters of Cassava Effluent and Surface Water of the contaminated Odo-Oba River

Physicochemical Parameters	water sample	Effluent	Permissible limit	Source	Health impact
Phenolpthalein Alkalinity	0.00	0.00		-	
Methyl orange Alkalinity	50.0±1.528[c]	20.0±0.577[a]		-	
Total Hardness	44.0±0.252[b]	52.0±0.300[a]	150	A	None
Calcium hardness (Ca^{2+}H)	28.0±0.252[a]	32.0±0.231[b]			
Magnesium hardness (Mg^{2+}H)	16.0±0.100[b]	20.0±0.100[c]		-	
Calcium (Ca^{2+}T)	11.2±0.058[a]	12.8±0.200[b]		-	
Magnesium total (Mg^{2+}T)	6.8±0.100[c]	7.6±0.0580[a]	0.2	-	Consumer-acceptability
Carbon dioxide (CO_2)	10.0±0.100[c]	23.0±0.681[b]		-	
Chloride (Cl^-)	12.0±0.000[a]	19.0±0.115[c]	250	A	None
Iron ($Fe^{2+)}$	0.6±0.015[c]	1.4±0.076[b]	0.30	A	None
Cupper ($Cu^{2+)}$	0.05±0.010[c]	0.25±0.010[c]	1.00	A	Gastrointestinal disorder
Manganese (Mn^{2+})	0.3±0.058[a]	0.5±0.036[a]	0.20	A	Neurological disorder
Lead (pb^{2+})	0.15±0.003[b]	0.4±0.010[b]	0.01	A	Cancer, interference with vitamin D
Fluoride (F-)	0.04±0.006[c]	0.04±0.002[c]	1.50	A	Fluorosis, bones and teeth morbidity
Sulphate ($So_4{}^2$-)	13.5±0.065[c]	22.0±0.125[b]	100	A	None
Nitrate (N03-)	1.2±0.021[a]	4.3±0.351[a]	50	A	Cyanosis & asphyxia in infants under 3 months
Phosphate ($P0_{4)}$	0.25±0.030[b]	0.7±0.087[b]		-	
Sodium (Na^+)	1.1±0.208[a]	2.4±0.032[c]	200	A	None
Potassium (K^+)	0.8±0.015[c]	1.1±0.225[c]			
Total solids	254.0±0.500[a]	426.0±0.577[b]	500	A	None
Total dissolved solids	218.0±0.608[b]	362.0±0.115[c]		-	
Suspended solids	36.0±0.000[a]	64.0±0.306[b]		-	
COD	10.4±0.030[c]	11.6±0.120[a]		-	

Turbidity	8.5±0.021[b]	19.0±0.058[c]	5.00	A	None
PH	6.4±0.026[c]	5.3±0.010	6.5-8.5	A	None
Colour (murky)	15±0.100[a]	60±1.258[c]	15	A	None
Dissolved oxygen (1st day)	5.0±0.035[b]	0.4±0.025		-	
Dissolved oxygen (5th day)	1.4±0.010[c]	0.000[b]		-	
BOD	6.3±0.021[a]	7.4±0.125[c]		-	
Conductivity	89.04±0.012[a]	103.57±0.015[b]	1000	A	None
Silica ($Si0_2$)	8.0±0.293[c]	5.0±0.058[b]		-	
NH_3	0.056±0.002[c]	0.138±0.001[c]		-	
Cromium (Cr)	0.02±0.006[a]	0.05±0.001[a]	0.05	-	Cancer
Zinc (Zn)	0.8±0.021[b]	1.3±0.010[b]	3.00	A	None
Arsenic (AS)	0.00	0.00	0.01	A	Cancer
Aluminium (Al)	1.2±0.010[a]	2.0±0.212[c]	0.20	A	Potential neuro-degenrative disorders
Mercury (Hg)	0.00	0.00	0.001	A	Affect kidney and central nervous system
Cadmium (Cd)	0.00	0.00	0.003	A	Toxic to the kidney
Nickel (Ni)	0.00	0.00	0.02	A	Possible carcinogenic
Cyanide (CN)	0.08±0.006[b]	1.17±0.010[c]	0.01	A	Very toxic to the thyroid and the nervous system
Hydrogen sulphide (H_2S)	0.05±0.000[a]	1.3±0.032[c]	0.05	A	None
Odour	Fair	Repulsive	Unobjectionable	A	None

Values are expressed as mean ± SD from triplicates determinations, A= NSDWQ (2007) , conductance=μS/cm, Turbidity = NTU (Nephelometric turbidity unit), Colour =TCU (true colour units).Means on the same horizontal row between samples with same superscripts are not significantly different (p>0.05).

CHARACTERIZATION OF *Clarias gariepinus* LIVER RHODANESE FROM CASSAVA-EFFLUENT CONTAMINATED ODO-ODO OBA RIVER

From Fig. 2 below, the optimal pH for *Clarias gariepinus* liver rhodanese was 7.0. It is in harmonius with the pH of 8 reported by Okonji *et al*. (2011) and Yanhua *et al*. (2012) in mudskipper liver. The cyanide degrading enzyme from *Alcaligene*sp.DN25 with a narrow optimum pH range of 7.0-8.0 this value is smaller than the values which were found for Rainbow Trout 10.5 (Hossein and Reza, 2011), Mouse liver rhodanese 9.4 (Lee *et al*., 1995). However, it is above the values (6.0) for *Klebsiella edwardsii* reported by (Adedeji *et al*., 2017).

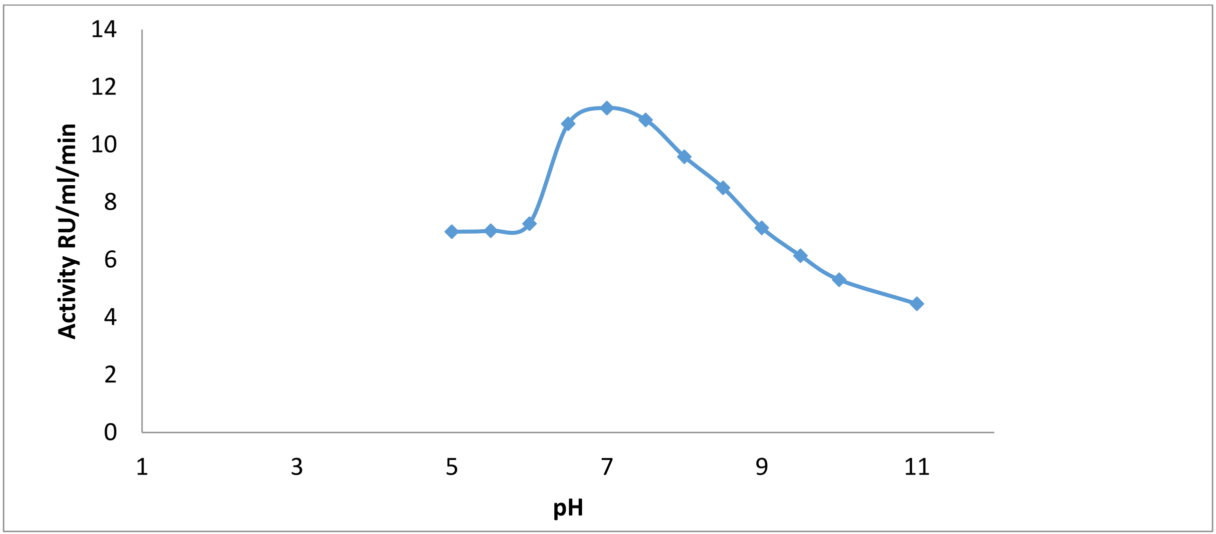

Figure 2. Effect of pH on Clarias gariepinus liver rhodanese from cassava-effluent contaminated Odo-Oba River

The optimal temperature obtained (Fig. 3) was 30˚C for rhodanese from the liver of the *Clarias gariepinus*, Odo-Oba River. This result is in harmonius with the results (30˚C) obtained by (Hossein and Reza, 2011) for the liver rhodanese of rainbow trout. The value of 40˚C for catfish liver rhodanese in Asejire Lake was reported by (akinsiku *et al*., 2010). The contamination level in water might likely leads to increase in various metabolic activities resulting in the release of heat.

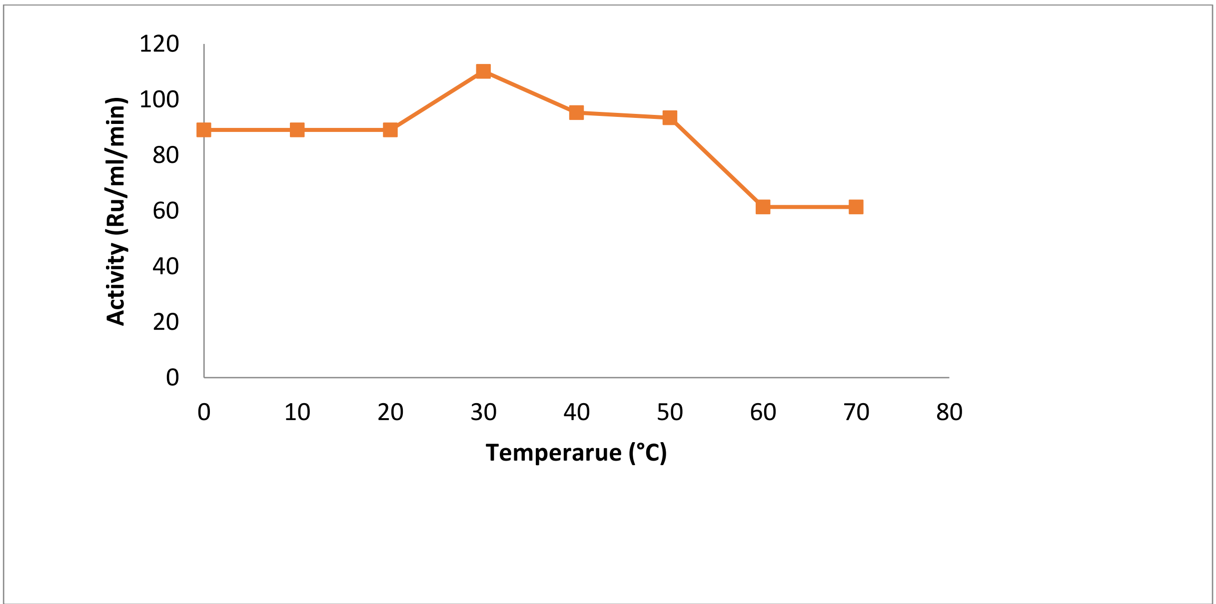

Figure 3. Effect of temperature on Clarias gariepinus liver rhodanese from cassava-effluent contaminated Odo-Oba River

In fig 4 and 5 the Vmax of 9.62 RU/Ml was obtained for the KCN substrate and 6.33 RU/Ml was obtained for the thiosulphate. While for the Km, higher values of 49.4 and 28.5 mM were obtained for the KCN substrate and $Na_2S_2O_3$ substrates respectively. However, the Km values of the fish liver

Rhodanese, indicated higher affinity for thiosulphate than for KCN as a substrate, although maximum activity was observed for KCN substrate. This observation is in agreement with the report of Adeyanju *et al.*, 2014 with Km values of 22.22mM(gut of earthworm) and 15.38 mM(body segment of the earthworm with 33.33 mM obtained for the KCN.

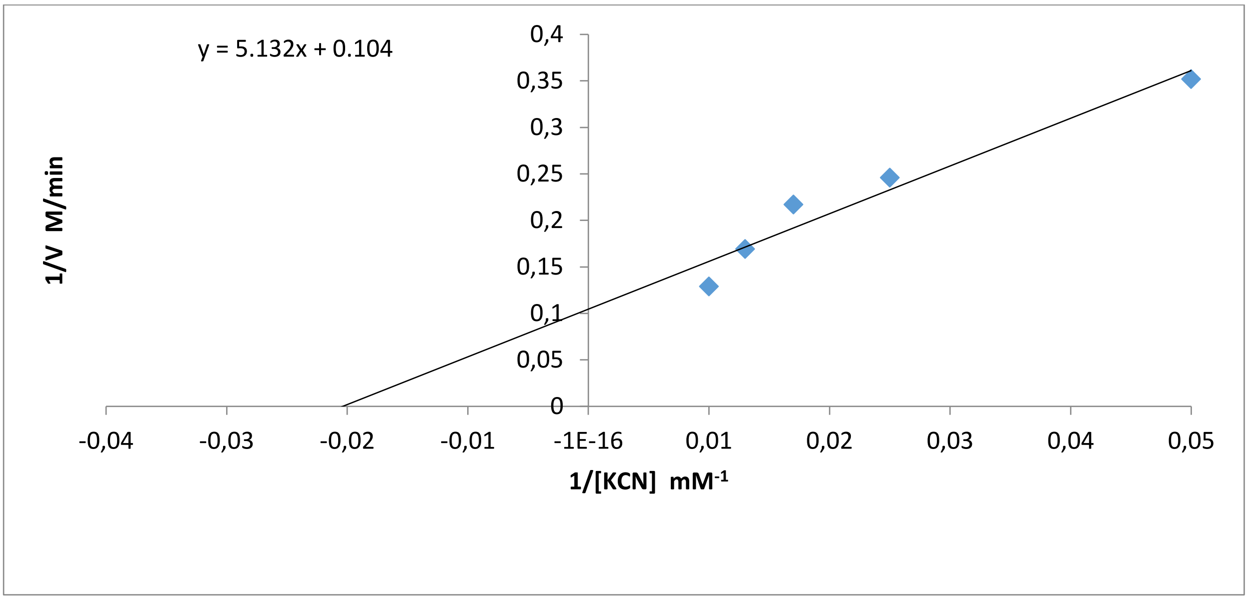

Figure 4. Lineweaver-Burk plot for the kinetic parameters of *Clarias gariepinus* liver rhodanese from cassava-effluent contaminated River. Enzyme was investigated by ranging the concentrations between 10mM and 100mM of KCN at a fixed concentration of 50 mM $Na_2S_2O_3$.

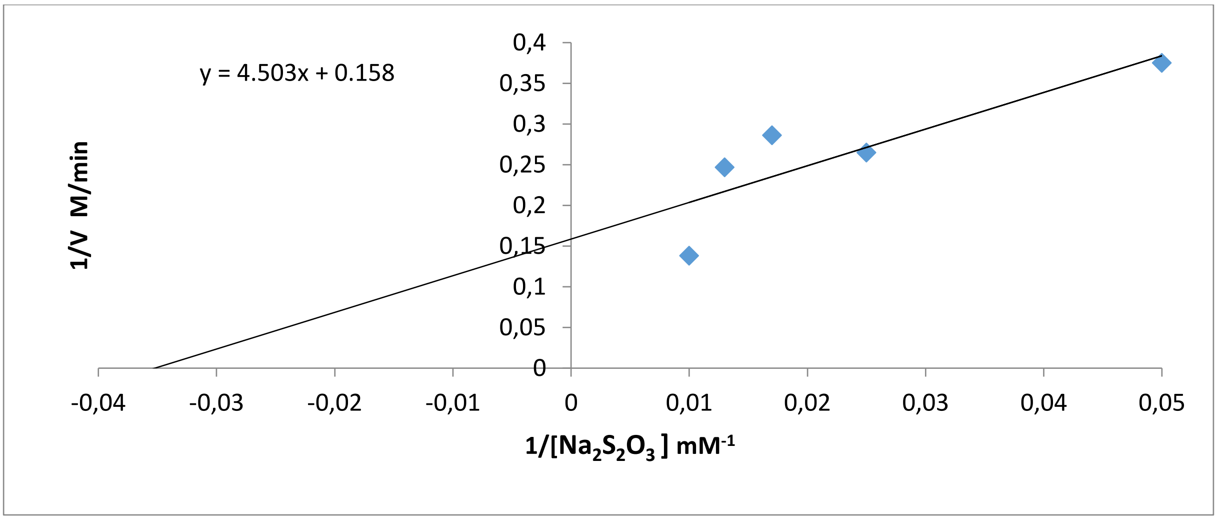

Figure 5. Lineweaver-Burk plot for the kinetic parameters of *Clarias gariepinus* liver rhodanese from cassava-effluent contaminated River. Ranging concentrations of $Na_2S_2O_3$ between 10mM and 100mM at a fixed 50 mM concentration of KCN.

Table 5. Kinetic Parameters of *Clarias gariepinus* liver rhodanese from cassava-effluent contaminated **Odo-Oba River Water**

Kinetic constants	KCN	$Na_2S_2O_3$
Km(mM)	49.4	28.5
Vmax (RU/Ml)	9.62	6.33

Table 6. Comparison of Km Values for *Clarias gariepinus* liver rhodanese from cassava-effluent contaminated Odo-Oba River with Other Isolated Liver Rhodanese Preparations from various cassava effluents contaminated sites

SUBSTRATE	Km(mM)						
	*Clarias gariepinus**	rainbow trout	Fruit bat	Mouse	Bovine	Human	Rat
KCN	49.4	36.81	13.36	12.5	19.0	9.5	NA
$Na_2S_2O_3$	28.5	19.84	19.15	8.3	6.7	4.5	4.4

*= present study, Data obtained from references (Hossein and Reza, 2011), NA, Data not available.

Table 7 below shows Inhibition studies on the enzyme with a number of chloride salts. The results indicated that the activity of the enzyme was not affected by Mg^{2+}, Mn^{2+},Ca^{2+} while Ba^{2+},Hg^{2+} and Cu^{2+} inhibited the enzyme considerably.

Table 7. Effect of cations on *Clarias gariepinus* liver rhodanese activity from cassava-effluent contaminated River

Salt	Enzyme Activity 0.5mM	Enzyme Activity 1mM
None	93±5.13	100±2.00
$MgCl_2$	84±1.16	82±1.00
$MnCl_2$	71±2.00	70±0.58
$CaCl_2$	69±0.58	65±1.53
$BaCl_2$	26±0.58	29±0.58
$HgCl_2$	36±0.55	38±1.00
$CuCl_2$	24±1.16	23±0.00

The Enzyme investigation was conducted using standard procedure of each salt at final concentrations of 0.5mM and 1.0 mM.

Conclusion

This study showed that cassava effluents contaminated soil and water samples in Odo-Oba environs. This is noticeable through the offensive odour observed in the area. The effluent increased the levels of Biochemical Oxygen Demand, Chloride, turbidity and total dissolved solids of the river. The effluent also impacted acidity to the soil by decreasing the soil pH. The results of heavy metal analysis showed elevated levels in the soils and surface water samples thus, posing threat to life in the area and therefore making the contaminated soil and water unsuitable for agricultural and home use. The result obtained for *Amaranthus spinosus showed* potential for phyto-extraction of heavy metals from the soil. In conclusion, rhodanese presence in *Clarias gariepinus* indicated high potential for cyanide detoxification safeguarding its survival in Odo-Oba River.

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