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STUDY ON THE EFFECT OF METALS CONCENTRATION ON BIOCHEMICAL PARAMETERS IN *OREOCHROMIS NILOTICUS* OF WARWADE RESERVOIR, DUTSE, JIGAWA STATE-NIGERIA

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ABSTRACT

Warwade water Reservoir and *Oreochromis niloticus*'s tissues (gills and liver) were evaluated by analyzing heavy metals concentrations and their effects on the oxidative stress enzyme from January to August 2022. Both field and laboratory assessments were conducted following established scientific protocols. Monthly sampling occurred between 6:00 – 7:00 am. Four stations denoted as A, B, C, and D, were chosen based on the diversity of anthropogenic activities surrounding the reservoir. The findings showed that heavy metals concentrations in the water ranked as follows: Chromium at 1.96mg/L, followed by Lead (1.74mg/L), Nickel (1.36mg/L), and Cadmium (1.03mg/L). Heavy metals values in fish tissues exhibited a significant decrease ($p<0.05$) in the following order for gill tissues (Pb > Cr > Ni > Cd) and liver (Pb > Cr > Cd > Ni). The recorded vlaues exceeded the recommended limits set by WHO (2021). Enzyme activities serving as oxidative stress biomarkers demonstrated a significant reduction ($P<0.05$) for Superoxide dismutase (SOD), Catalase (CAT) and Glutathione Transferase (GST), with higher mean activity observed in the gills with SOD (16.57 ±0.43), CAT (23.61±2.11) and GST (84.40±1.03) compared to liver samples of GST (81.10±0.51), SOD (14.32 ±1.08) and CAT (20.51±0.17) respectively. It may be inferred that the presence of metals in *Oreochromis niloticus* is a consequence of the discharge of pollutants into the water body, attributable to urbanization and the discharge of agrochemicals, which adversely impacted the water quality. Consequently, it is imperative to regulate uncontrolled discharges from human activities within the reservoir to mitigate the long-term degradation of this aquatic ecosystem.

Keywords: Antioxidants, Heavy metals, Cichlid fish, Toxicity, Warwade Reservoir, Jigawa State

INTRODUCTION

Water bodies are impounded with sole aim of water provision for many purposes including agricultural activities, drinking, and recreation among many purposes (Oladeji, 2020). Pollution of water bodies have been a contentious issue globally due to the rapid increase in industrialization, agrochemicals input, electronic-waste and domestic waste release into the water bodies leading (Saiyadi *et al*.., 2022). Harmful effect of the detrimental effects of metals on organisms have been documented, arising from effluent's dis, municipal, and urban runoff wastewater (Garba *et al*.., 2023). As a results of their inherent toxicity, bioaccumulative characteristics, and non-biodegradable nature in aquatic ecosystems, metals represent a significant category of aquatic contaminants (Sani *et al*.., 2020). The adverse impacts on aquatic resources are often contingent upon the solubility of the compounds, duration of exposure, permeability within cellular compartments, mobility, and concentrations, ultimately affecting physiological functions (Akinwande *et al*.., 2016).

Moreover, metal pollution in aquatic environments were reported to induce detrimental impact on organisms as a results of their bioaccumulative properties across the food chain (Butu *et al*.., 2019). A number of water bodies in Nigeria were reported to contain high heavy metals concentrations such as Cr, Ni, Pb, Mn, Co, and Cd, originating from human activities such as industrial discharges among other factors (Abdullahi *et al*.., 2021). Fish serve as effective indicators for monitoring water quality and act as bioindicators of pollution (Akinwande *et al*.., 2016). Heavy metals concentrations along with other pollutants were reported to initiate catalytic reactions in fish tissues producing reactive oxygen species (ROS), causing oxidative stress (Ullah *et al*.., 2019). Imam (2012), Saiyadi *et al.* (2022) documented elevated values of metals , specifically Cr, Cd, Cu and Pb, that exceeded the standards established by the WHO (2021) in Wasai, Kafinchiri, and Warwade Reservoirs, respectively. In light of the aforementioned findings, this study was designed to evaluate the heavy metals level in fish and water tissue of Warwade water body, Jigawa State, Nigeria.

MATERIALS AND METHODS

This research was carried out at Warwade water body, situated approximately some kilometers south of Dutse, Capital of Jigawa State within the following coordinates: 11° 45' 0" North and 9° 13' 0" East (Figure 1). The reservoir is fed by three significant tributaries, namely Dutse, Tsangaya from Jigawa State, and Albasu local government from Kano State. The geological composition contains mainly sedimentary formations of the Chad Lake. The region experiences wet and dry seasons characterized by a prolonged summer and a brief winter, with an annual mean temperature of 31°C. The reservoir features a crest height of 10 meters, a crest length of 2780 meters, and a reservoir capacity of 429.5 million cubic meters with a spillway of box culvert design. The local populations, primarily comprising farmers and fishers, rely heavily on this water body as a vital source of livelihood, often leading to the over-exploitation of its resources.

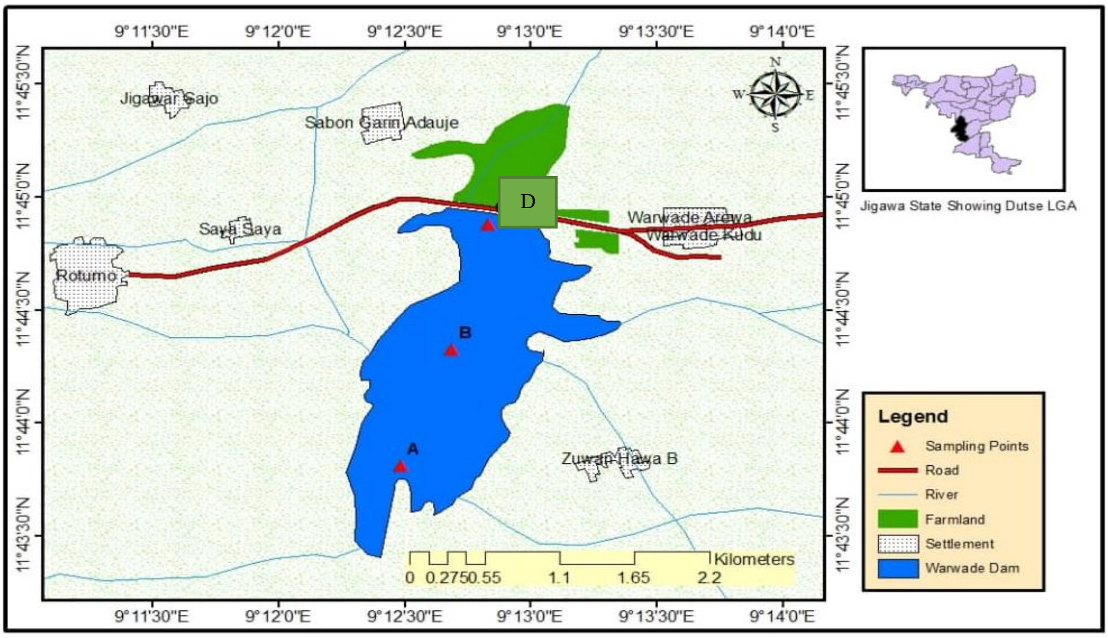

Figure 1: Map of Warwade Reservoir Showing Sampling Sites. (Cartography Lab. Geography Department BUK (2022)

Sample Collection

Water and fish sampling were executed on a monthly basis over an eight-month duration, conducted in triplicate from 07:00 am to 07:30 am at each designated sampling site. Four sampling locations, denoted as A, B, C, and D, were chosen based on the varying degrees of human activity surrounding the water body. Fish were collected by utilizing gill nets, traps, and hooks, with assistance from local fishermen, as outlined by Badamasi (2014). The identification of fish species was facilitated through the use of a standardized identification guide. Olaosebikan and Raji (2004).

Water samples were procured in opaque 1-liter containers from the three designated sampling locations. The Cd, Cr, Ni, and Pb concentrations were assessed in accordance with the methodology adopted by Nsofor *et al.* (2014). Precisely hundred (100mL) of each water collected was transferred into 250ml beaker. An aliquot of 10 mL of HNO_3 was introduced and subjected to heating at 100°C. Samples were allowed to evaporate to approximately 60-70 mL, followed by the addition of an additional 5 mL of HNO_3 with continuous heating until a final volume of 20 mL was achieved. The samples underwent filtration, diluted with water to 50 mL. Heavy metals concentrations were quantified by utilizing Perkin Elmer PinAAcle 900H Model atomic absorption spectrophotometer at the Central Laboratory, Bayero University, Kano.

Oreochromis niloticus collected were analyzed for the presence of heavy metals in liver and gill tissues using protocol recommended by Garba *et al.* (2023). The fish samples were processed with a plastic knife, and the gill and liver tissues were desiccated in an oven at approximately 105ºC for a duration of 24 hours. Following the drying process, the samples were milled into powdered form. The resulting powder was preserved until the digestion process. Precisely 2.0 g of the powdered samples were transferred into Teflon containers designated. An aliquot of seven (7 mL) of nitric acid, 2 mL of H_2O_2, and 1 mL of $HCLO_4$ were incorporated into the sample; the rotor for digestion was then positioned within the microwave digestion system and programmed according to the animal tissue protocol. Upon the completion, the digested samples were filtered and diluted with distilled water to 50 mL. Heavy metals concentrations were quantified by utilizing Perkin Elmer PinAAcle 900H Model atomic absorption spectrophotometer at the Central Laboratory, Bayero University, Kano.

Oxidative Stress Enzymes Examination

The analysis of antioxidant enzymes was conducted in accordance with the methodology delineated by Mandeep and Rajinder (2017). SOD (BC0170), CAT (BC0200), and GST (Cat No. BC1190) kits produced by Solarbio Life Science were used for the analysis in accordance with the manufacturer's directives. The Fish organs were meticulously excised and washed in 5 mL BPS isolation buffer to reduce blood stains. Organs were milled and ground with a mortar and pestle to obtain homogenate. Homogenate was subjected to centrifugation at 12,000 g for five minutes to reveal the cellular components. Antioxidant enzymes activity of each organ was quantified utilizing a Spectrophotometer (UV-6300PC double beam model) at Biochemistry Department of Bayero University, Kano.

Bioaccumulation Factor (BAF) Determination

Heavy metals Bioaccumulation in fish muscle is delineated according to the guidelines established by the United States Environmental Protection Agency, 2014).

$$BAF = \frac{M\,tissue}{M\,water}$$

Where; M (tissue) denotes concentration of metals in fish tissue expressed in mg/kg, and M water represents the concentration of metals in water quantified in mg/L.

Statistical Analyses

The dataset was subjected to descriptive statistical analysis to ascertain means and standard deviations. Furthermore, the data underwent one-way analysis of variance (ANOVA) to identify variations among sites, and where significant differences were detected, they were delineated using Least Significant Difference (LSD) at a significance level of 0.05%. Seasonal fluctuations were evaluated employing Student's independent T-test. All statistical analyses were conducted utilizing SPSS software version 20.0.

RESULTS AND DISCUSSION

Table 1 elucidates the sites differences of metal concentrations in water sampled from Warwade water body. The results demonstrated that lead (Pb), chromium (Cr) and nickel (Ni) exhibited highest mean concentrations of 1.74, 1.96 and 1.36 mg/L, respectively. The heavy metals concentrations in water samples indicated a statistically significant decrease ($p<0.05$) in order of Cr > Pb > Ni > Cd. Statistically, the concentrations exhibited difference ($p<0.05$). Concentrations of metal varied spatially ($p<0.05$).

Table 1: Sites Variation of Heavy Metals Concentrations in Water Sampled from Warwade Reservoir, Jigawa State, Nigeria

Parameters	site A	Site B	Site C	D	Mean	FAO/WHO (2018)
Cr (mg/L)	1.16±0.02^{a}	0.21±0.08^{a}	0.31±0.01^{a}	0.31±0.06^{a}	**1.96**	2.0
Pb(mg/L)	0.55±0.01^{a}	1.00±0.01^{a}	0.12±0.01^{a}	0.13±0.10^{a}	**1.74**	0.05
Ni (mg/L)	0.45±0.10^{a}	0.39±0.01^{a}	0.70±0.01^{a}	0.26±0.00^{a}	**1.36**	0.001
Cd(mg/L)	0.001±0.01^{b}	0.13±0.00^{a}	0.62±0.01^{b}	0.49±0.03^{b}	**1.03**	0.05

Table 2 presents the mean heavy metals concentrations recorded in Warwade reservoir with respect to seasonal changes. Seasonally, the mean values differed significantly ($P<0.05$).

Table 2: Seasonal Variation of Mean Heavy metals Concentrations in Water from Warwade Reservoir

Season	Heavy metals			
	Cr(mg/L)	Cd(mg/L)	Ni(mg/L)	Pb(mg/L)
Dry	1.020±0.01^{b}	0.001± 0.010^{a}	1.009± 0.001^{a}	0.620± 0.010^{a}
Wet	1.409 ± 0.01^{a}	0.0020± 0.100^{a}	1.110± 0.001^{b}	0.908±0.001^{a}

Note: mean followed by superscript with different letters across the column are significant at $P<0.05$ using LSD

Table 3 delineates the variations in the concentrations of Ni, Cd, Cr, and Pb in the organs of *O. niloticus*. Mean Cd concentration was spatially elevated in the liver at sampling site A, measuring 0.78 mg/kg, in comparison to the liver from site A with 0.56 mg/kg. The mean Cd concentration did not differ spatially ($p>0.05$). Nickel exhibited the elevated mean value in the liver tissues at site D, recorded at 1.95 mg/kg, followed by site A at 1.65 mg/kg, with the reduced value documented in the liver at sampling site C, which was 0.51 mg/kg. As can be observed spatially, concentrations of Cd and Cr differed considerably ($p<0.05$).

Table 3: Sites Difference of Heavy Metal concentrations in *O. niloticus* Collected from Warwade Reservoir

Parameters/ tissues (mg/g)		Site A	Site B	Site C	Site D	FAO/WHO (2018)
Cr (mg/kg)	Liver	0.42±0.00^{a}	1.03±1.01^{a}	0.81±0.01^{a}	1.03±1.01^{a}	0.05
	gills	0.76±0.16^{a}	0.51±0.00^{a}	0.60±0.12^{b}	0.71±0.00^{a}	
Ni (mg/kg)	gills	1.43±0.11^{ab}	1.65±0.01^{b}	1.15±0.06^{a}	1.95±0.01^{bc}	2.0
	liver	0.55±0.21^{a}	0.83±0.01^{a}	0.51±0.06^{a}	0.83±0.01^{a}	
Pb (mg/kg)	Liver	0.46±0.01^{ac}	0.88±0.01^{b}	0.61±0.012^{a}	0.06±0.01^{a}	0.01
	gills	0.87±0.12^{b}	0.95±0.01^{b}	0.84±0.71^{b}	0.51±0.01^{a}	
Cd (mg/kg)	Liver	0.56±0.03^{b}	0.65±0.00^{b}	0.71±0.01^{b}	0.71±0.00^{ab}	0.05
	gills	0.78±0.04^{b}	0.78±0.01^{a}	0.71±0.01^{a}	0.30±0.001^{a}	

Values are expressed as mean and SD; means with differing superscripts within a row exhibited significant differences ($P<0.05$).

Table 4 revealed the mean concentrations of metals recorded in *O. niloticus* sampled from Warwade reservoir with respect to seasonal changes. Seasonally, the mean values differed significantly ($P<0.05$).

Table 4: Seasonal Difference of Heavy Metals Concentrations in *O. niloticus* Sampled from Warwade Reservoir

Parts	Season	Heavy metals			
		Cr (mg/kg)	**Cd (mg/kg)**	**Ni (mg/kg)**	**Pb (mg/kg)**
Liver	Dry	0.850^{a}	0.010^{a}	0.43^{a}	0.011^{a}
	Wet	0.780^{a}	0.021^{a}	0.64^{a}	0.031^{a}
Gills	Dry	0.224^{a}	0.018^{a}	0.834^{a}	0.320^{a}
	Wet	0.574^{a}	0.394^{a}	0.990^{a}	0.705^{a}

Note: means followed by different superscript alphabets across the column differed significantly ($P<0.05$)

Mean sites difference of antioxidant enzymes activity tissues (gills and liver) of *O. niloticus* of Warwade Reservoir is presented in Table 5. The results revealed significant difference (p<0.05) in SOD, CAT and GST activities.

Table 5: Spatial variation of Oxidative Stress Enzymes activity in Warwade reservoir

Organ	Site	GST (nmol/min/mg prot)	SOD (unit/ mg prot.)	CAT (Unit/mg prot.)
	A	76.80 ±0.01[b]	11.41 ±0.01[a]	17.21±2.10[a,b]
Liver	B	82.4 ±1.03[c]	12.27±0.17[a]	23.81±1.05[a,b]
	C	88.3 ±0.01[a]	17.74 ±0.01[a]	17.54±0.13[a]
	D	81.50 ±0.01[a]	17.98 ±0.12[b]	24.90±0.01[a]
	A	94.1 ±0.21[a]	19.53 ±0.22[b]	19.56±0.01[b]
Gills	B	81.4 ±0.01[a]	15.65±0.78[a]	27.32±1.5[a]
	C	78.3 ±0.11[a,b]	15.80 ±0.07[a]	21.65±0.54[a]
	D	83.10 ±0.93[a]	17.71 ±0.91[a]	25.45±1.03[b]

Note: means followed by different superscript alphabets across the column differed significantly (p<0.05)

Discussion

In the present finding, concentrations of heavy metals in water and fish tissues revealed that most of the studied parameters were above the recommended limit of FAO/WHO (2018). The situation is attributed to the rapid population growth, increase urbanization, electronic waste deposition among other natural factors (Garba *et al*.., 2023). Variations among sampling sites indicated that chromium concentrations in water exhibited the highest mean values, while cadmium recorded the lowest concentrations. The elevated concentration of Cr is attributed to the deposition of garbage from domestic activities comprising automobile deposition, empty paint containers, smelters and local tanneries. This observation is in line with the results reported by Saiyadi *et al.* (2022). Furthermore, it might ascribed to the geologic pattern of minerals which vary from one site to another (Alzahrani *et al.*, 2023). Sites variation analysis revealed that the concentration of Cr at site C surpassed the recommended for freshwater, as stipulated by the WHO (2021), which is set at 1.0 mg/L. The heavy metals values within the water samples decline in the following order: Cr > Pb > Ni > Cd, which corroborates the findings by Saiyadi *et al.* (2022) in the same reservoir, who reported elevated Cr concentration in water of 1.51 mg/L, exceeding the WHO limit (2021) of 1.0 mg/L. Site variations demonstrated elevated mean Cr values of 1.96 mg/L, potentially attributed to heightened nutrient input from runoff and agrochemical contributions from adjacent agricultural land, as noted by Shawai *et al.*. (2019). It could also be influenced by variation in mineral composition within the sampling area.

Lead exhibited the elevated concentration in the gill tissues at sites B and C. The elevated values of Pb is attributed to the direct contact with waste water as reported by Abdullahi *et al.* (2021) in the Jakara River, Kano, Nigeria. Additionally, this could be a consequence of the substantial discharge of domestic waste into the reservoir. Release of agrochemicals such as pesticides and fertilizers into the reservoir might have contributed to elevated concentrations of lead (Pb). A comparable finding regarding the elevated levels of Pb was documented by Mustapha *et al.* (2013) Jakara reservoir. The peak Pb concentration of 0.91 mg/kg in gill surpasses the 0.57 mg/kg obtained by Ibrahim and Said (2010). The concentration of Pb measured in the fish tissues exceeded the recommended threshold of 0.01 mg/kg established by the World Health Organization (WHO, 2021).

Nickel (Ni) exhibited the elevated mean concentration in gills at site C, whereas the lowest concentration was observed in liver tissues at site A. The elevated levels of Ni detected in the gill tissues might be ascribed to the organ's metabolic functions, cellular activities among other critical roles in the bioaccumulation process. This observation aligns with the findings of Butu *et al.* (2018). Furthermore, the nature of Ni and the chemical conditions of the water might serve as additional factors influencing the accumulation process (Samson *et al*.., 2020). The concentrations of copper (Cu) across all examined tissues remained below the recommended of 0.01 µg/g as stipulated by the WHO (2021). The recorded values were higher than the 0.04 µg/g and 1.12 µg/g observed in gills and liver, respectively, as reported by Ahmad *et al.* (2018) from Kafinchiri Reservoir, Kano.

Chromium, in minute amounts, facilitate metabolism of cholesterol in a cell; however, at elevated concentrations, it has been reported to be harmful to aquatic organisms (Adamu *et al*.., 2016). Elevated concentration found in the examined fish tissues (gill) might result from their proximity to a polluted environment, causing high deposition compared to the liver (Friday *et al*.., 2013). The chromium detected might originate from local dying and tanning activities along the water channel, as indicated by Shawai *et al.*(2018). Chromium concentration in *Oreochromis niloticus* is attributed to the predatory habit behaviors of the fish, indicating metal bioaccumulation at the climax trophic levels. Concentrations of Cd in water samples recorded a mean of 1.03 mg/L, which exceeds the recommended of 0.01 mg/Kg set by the WHO (2021). The measured concentration of cadmium is attributed to the morphological nature of the sampling area as recorded by Jeyakumar *et al.* (2023). Cadmium is a hazardous metal which produce negative effects on fish physiology, especially on its reproduction (WHO, 2021). It is an industrial contaminant that causes harmful effects to vital organs in living organisms (WHO, 2021).

The heavy metal in water during the study period exhibited this trend in concentration Cr> Cd>Pb>Ni. The difference in the metals concentrations might be due to contact of water with rock and soil containing large deposits, increased urbanization and industrialization. Similar observations were reported by Doka *et al.* (2020). Presence of these metals in the fish dependent on bio-availability in water and sediments (Zhou *et al*.., 2019) as well as dietary intake. From the presence study, concentration of heavy metals in fish tissues revealed a decrease in the order of decreasing concentration in gill tissues (Pb > Cr > Ni > Cd) and liver (Pb > Cr > Cd > Ni). The affinity of various metals at fish tissues varied due to proximity of the tissues and bioaccumulative capacity of the metals such as Ni, Cd, Pb which play vital roles in metabolism. Heavy metals like copper and Mn function as a cofactor in many enzymatic processes while iron is entails with haemoglobin production in the blood of fish. This is in consistent with the finding of (Maitera *et al*.., 2010).

Superoxide dismutase (SOD) prevent the body from the oxidative stress in biota is called (Oluwatosin *et al*.., 2016). It

alters radicals of superioxide in the mitochondria and peroxisomes into H_2O_2, which later transformed by CAT into non-reactive oxygen and water (Oluwatosin *et al.*., 2016). The sampled fish during the research were recorded to have high GST, SOD and CAT activity. The high activity in the fish tissues is due to production of oxy-radical by dissolved mineral contents in the water. Similar observation was reported by Ezenwosu *et al.* (2021). Superoxide radical production by high SOD and CAT activity, results to a feedback mechanism processes by contaminants in the water which might be the leading cause for the high activity recorded in the present study.
The present study showed a higher activity of GST in all the examined fish tissues which might be as results of pollutants introduction into the water body where the fish survive. This is line with the finding of Akinwande *et al.* (2016). GST concentrations increased in fish tissues (gills and liver) is attributed to the availability of pollutants in the water reservoir stimulating GST activity as a result of pollutants discharge into the water course. This observation is similar with the finding of Ullah *et al.* (2014). Ezenwosu *et al.* (2020) reported that fish are prone to heavy metals contaminants which serve as biomarkers for aquatic ecosystem assessment.

CONCLUSION
The findings revealed that heavy metal concentrations in the water ranked as follows: Chromium at 1.96mg/L, followed by Lead (1.74mg/L), Nickel (1.36mg/L), and Cadmium (1.03mg/L). Heavy metals concentrations in fish tissues exhibited a significant decrease ($p<0.05$) in the following order for gill tissues (Pb > Cr > Ni > Cd) and liver (Pb > Cr > Cd > Ni). The recorded values exceeded the recommended limits set by WHO (2021). Enzyme activities serving as oxidative stress biomarkers demonstrated a significant reduction ($P<0.05$) in SOD, CAT and GST, with higher mean activity observed in the gills with SOD (16.57 ±0.43), CAT (23.61±2.11) and GST (84.40±1.03) compared to liver samples of GST (81.10±0.51), SOD (14.32 ±1.08) and CAT (20.51±0.17) respectively. It may be inferred that the presence of heavy metals within fish tissues is a consequence of the discharge of pollutants into the water body. It is therefore recommended that uncontrolled release from human activities within the reservoir should be minimized in order to curtail degradation of the reservoir in the long run.

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