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Effects of Heavy Metals Pollution on Some Fish and Mollusc Species from Port Sudan, Sudan

Issra Mohammed Osman Mahjoub[1, *], Mohamed Elamin Hamza[2, 3]

[1]Department of Fisheries, College of Natural Resources and Environmental Studies, University of Bahri, Khartoum, Sudan
[2]Department of Chemistry and Biology, Faculty of Science and Technology, Merowe University of Technology, Merowe, Sudan
[3]Institute of Marine Research, Red Sea University, Port Sudan, Sudan

Email address:
israaosman80@hotmail.com (I. M. O. Mahjoub), Maelsaimhu7@gmail.com (M. E. Hamza)
[*]Corresponding author

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Abstract: The effects on fishes Lethrinus harak and Cephalopholis minata and on the mollusc Tridacna maxima exposed to different concentrations of heavy metals vanadium, nickel, and copper were investigated. The study indicated that the LC50 for nickel were 198.200 ppm, for L. harak, 196.041 ppm for C. minata and 198.200 ppm for T. maxima. The LC50 for copper were 197.175 ppm for L. harak, 272.932 ppm for C. minata and 272.841 ppm in T. maxima. However, LC50 for vanadium recorded 131.836 ppm for L. harak, 164.769 ppm for C. minata and 164.037 ppm for T. maxima. On the other hand, LT50 due to nickel exposure recorded 74.815, 47.963, and 95.116 hours, for L. harak, C. minata and T. maxima, respectively. LT50 due to copper recorded 35.041, 47.681, and 71.835 hours for L. harak, C. minata and T. maxima, respectively. However, LT50 for vanadium were 11.989, 47.511 and 5.792 hours for L. harak, C. minata and T. maxima, respectively. In this study no response was detected in lower concentrations of nickel and copper i.e. 4 ppm and 32 ppm, however a high response was detected with the same concentrations of vanadium. The study indicated that T. maxima, was more tolerant for heavy metals pollution than L. harak and C. minata. Nickel concentrations detected in tissues analysis were 0.561-0.04 ppm, 0.421-0.02 ppm and 0.871-0.03 ppm for L. harak, C. minata and T. maxima, respectively. While copper concentrations recorded 1.1030-0.09 ppm, 0.4060-0.02 ppm and 1.35-0.03 ppm for L. harak, C. minata and T. maxima, respectively. However, vanadium concentrations, recorded 0.010-0.00 ppm, 0.04-0.014-0.01 ppm and 0.042-0.00 ppm for L. harak, C. minata, and T. maxima, respectively.

Keywords: Heavy Metals, Fish, Mollusca, Pollution, Red Sea, Sudan

1. Introduction

Marine pollution is a global environmental problem mostly attributed to human activities on land, water and air. Sediments and organisms releasing potentially toxic substances into the water also contribute to the level of contamination. Contaminants can stay in the water in dissolved form or they can be removed from the water column through sedimentation [1]. On the other hand oil pollution was dangerous from its discovery throughout stages of production, transportation, refining, processing, storage, marketing and even disposal of used products. This was resulted in bioaccumulation and physical contamination of beaches. The seas and oceans were polluted by millions of tons of oil annually since most of the factories and refineries were built along the coast [2]. In Japan, as a result of discharge of waste water containing mercury in Port Minamata bioaccumulation in tissues of marine organisms occurred and led to cases of poisoning in 1952 known as Minamata syndrome [3]. The marine biota and habitat of Port Sudan area were negatively affected by discharge of harmful substances into the marine environment or indirectly from land based activities [4]. However, assessment and effects of heavy metals pollution at the Sudanese Red Sea coast were also studied [5, 6].

Sudan has become an oil exporting country since 1999 and two ports (Bashayer I and Bashayer II) were constructed for oil export and import. Approximately 400,000 barrel were exported per day since the mid of 2006. However, few and

small oil spills were often recorded in the area within and adjacent the port (Ministry of Oil, personal communication). Many scientists have designed methods for measuring lethal levels of pollutants for aquatic organisms, thus, nearly all the acute lethal actions ceased were within four days and half-lethal concentration LC50 [7, 8].

Major chronic inshore marine pollution problems can often be attributed to the discharge of large volumes of wastes that have local impacts. These include materials which are partially biodegradable, such as raw sewage sludge, food and beverage processing work, pulp and paper mill effluents, woolen and cotton gin waste, and sugar refinery effluents, also solid wastes were in this category [9]. The area around Port Sudan was polluted, being a harbour for oil and gas product export and import, in addition to impacts of the existing industrial areas in Port Sudan town. Similarly the atmosphere near the power plant station was polluted with vapors and gases emitted by power generators, and black fumes are seen at the time of generators operation. Also the traffic participates in atmosphere pollution, which ends at the marine environment. Oil film was also seen covering part of water surface in this site, which was regarded as a nursery ground. However fingerlings were seen in the tidal area, in spite of oil film in Alkheir oil terminal (refined products), the area was exposed to oil pollution through handling, spills from tankers, ballast waters, etc. The Red Sea is considered as semi-enclosed area that exposed and threatened by pollution, stated that due to its relatively small size, limited oceanographic circulation and high endemism, the Red Sea is particularly vulnerable to pollution, loss of species, and reduction in ecosystem productivity. Accidents may happen during oil import and export operations [10]. Oil contains high level of heavy metals (according to analysis of Petroleum Central Laboratory). Since these heavy metals enter the water it was well expected to find its way to aquatic organisms.

2. Material and Methods

2.1. Experiment Setup

Over 50 live specimens from each of fishes Lethrinus harak and Cephalopholis minata and Mollusca Tridacna maxima were collected by local fishers form Abu Hashish area in Port Sudan, Red Sea State -Sudan. The specimens were then transferred to the Red Sea Fisheries Research Centre laboratory, kept in aquariums and were acclimatized to laboratory conditions. Deformed specimens or that showed abnormal behaviour were instantly rejected. According to modified bioassay methods of a pilot experiment was setup with heavy metals pollutants, vanadium: ammonium vanadate NH_4VO_3 [8, 11]. Copper: cupric nitrate trihydrate. Copper (II) nitrate trihydrate $Cu(NO_3)_2.3H_2O$ and nickel: nickel nitrate hexahydrate, $Ni(NO_3)_2.6H_2O$, the concentrations prepared for each metal were: 273 ppm, 200 ppm, 165 ppm, 132 ppm, 100 ppm, 64 ppm, 32 ppm, 16 ppm and 4ppm. The pollutants were dissolved in seawater and eight specimens per concentration were tested. The experiment set-up was consisted of nine aquariums each containing 32 liters vigorously aerated sea water (100% saturation) and were left at room temperature (22°C - 28°C) and diffused day light. Identified weights of fishes and mollusc specimens (50–70 gm.) were selected. Eight samples from each species were introduced into the experimental system 30 minutes after the introduction of pollutants; an identical control system with pollutant-free water was maintained parallel to each experiment. Water in both systems was regularly aerated for one hr. every 12 hrs to avoid acute depletion of dissolved oxygen. All experiments were carried out in triplicate with the regular feeding for both the experimental and control animals. Observations on L. harak, C. minata and T. maxima mortality were recorded every 3, 6, 12, 24, 36, 48 and 72 hrs. Dead specimens were preserved in 10% formalin for pathological investigation and for Atomic Absorption Spectrophotometer (AAS) analysis. Concentrations of three heavy metals V, Ni and Cu in tissues of fishes and mollusc specimens were determined using (AAS) following [12].

2.2. Effects of Some Heavy Metals on the Mortality Rate of Specimens

The effects of exposure of T. maxima, L. harak and C. minata to different concentrations of vanadium, nickel and copper were studied with special reference to their survival (table 1, 2 and 3) survival or reciprocal “mortality” was studied in terms of half-lethal concentration (LC50) and half-lethal time (LT50) for the experimental samples (Figures 1, 2 and 3). The mortality values were calculated directly using plots of a cumulative response against pollutants concentration, time of exposure logarithmic-probability paper and from semi logarithmic regression analysis of the same parameters. Cumulative response (%) when regressed against pollutant concentration (ppm) and lethal time in hours resulted in highly significant predictive equations. The variations of the LT50 for different concentration of pollutants used were presented by histogram for all experimental fish species and mollusc, when the LT50 were plotted against the logarithms of pollutants concentrations (Figures 1-9) and progressive experimental decrease occurred with increase in concentration for both fish and mollusc populations studied.

3. Results and Discussion

3.1. Effects of Vanadium on the Mortality Rate

The results obtained from this work indicated that the LT50 and LC50 values were low at vanadium treatment, thus, values of LT50 were 11.989 hours, 5.792 hours and 47.511 hours for L. harak, C. minata and T. maxima, respectively, while that of LC50 were 131.836 ppm, 164.769 ppm and 164.037 ppm for L. harak, C. minata and T. maxima, respectively (Table 1, Figures 1, 2 and 3). The correlation coefficient between concentrations and mortality was

significant ($p<0.05$) for T. maxima and L. harak, thus, the correlation was very highly significant for C. minata ($p<0.001$).

Table 1. *Probit analysis of fishes, and mollusc, correlation between concentration (Y) and mortality (X).*

Probit analysis*	Tridacna maxima	Lethrinus harak	Cephalopholis minata
LC50	164.037	131.836	164.769
LT50	47.511	11.989	5.792
MCR	18.23	24.67	12.04
Correlation coefficient (r)	0.71	0.57	0.86
Regression equation Y=a+ b In X	Y=5.212-0.283 In X	Y=5.492-0.406 In X	Y=7.632+0.625 In X
Correlation significance	0.027	0.041	0.001

* Calculated according to method of Finney (1971).

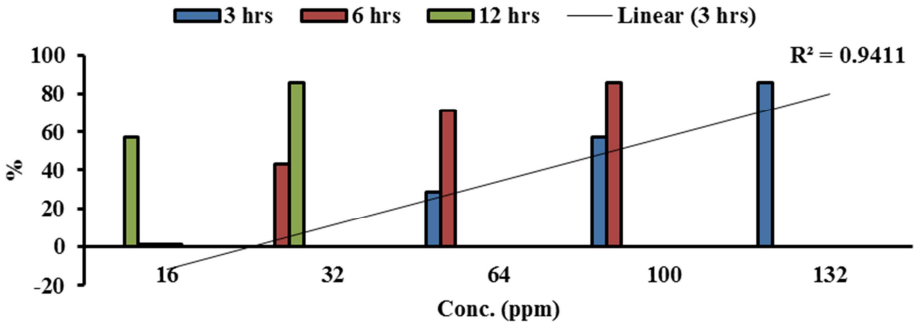

Figure 1. *Vanadium effect on Lethrinus harak.*

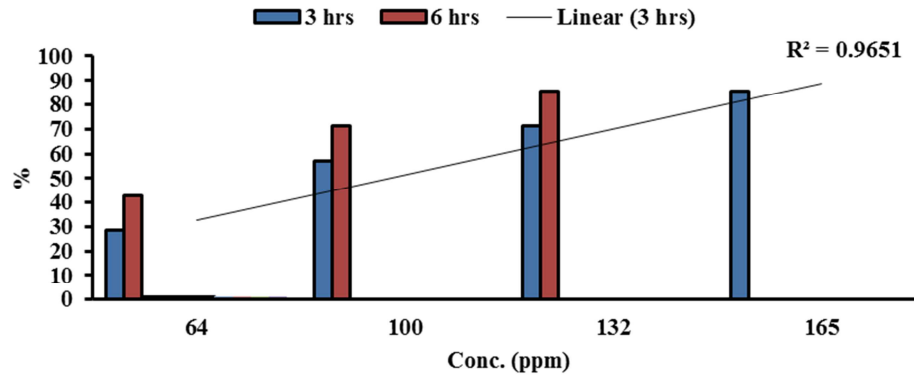

Figure 2. *Vanadium effect on Cephalopholis minata.*

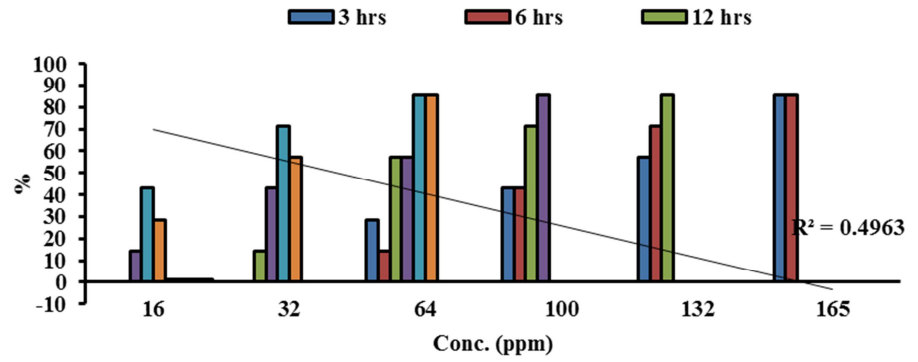

Figure 3. *Vanadium effect on Tridacna maxima.*

3.2. Effects of Nickel on the Mortality Rate

Values of LT50 recorded at nickel treatment were 47.815 hours, 47.963 hours and 95.216 hours for L. harak, C. minata and T. maxima, respectively, while that of LC50 were 199.236 ppm, 196.041 ppm and 198.200 ppm for L. harak, C. minata and T. maxima, respectively (Table 2, Figures 4, 5 and 6). The correlation coefficient between concentrations and mortality was extremely highly significant ($p<0.000$) for C. minata and highly significant ($p<0.01$) for T. maxima and insignificant ($p>0.05$) for L. harak (table 2).

Table 2. *Probit analysis of fishes, and mollusc, correlation between concentration (Y) and mortality (X).*

Probit analysis*	Tridacna maxima	Lethrinus harak	Cephalopholis minata
LC50	198.200	199.236	196.041
LT50	95.216	47.815	47.963
MCR	19.25	26.38	11.09
Correlation coefficient (r)	0.72	0.69	0.81
Regression equation Y=a+ b ln X	Y = 4.069+ 0.549 ln x	Y = 4.9922+ 0.1793 ln x	Y = 4.8796+ 0.3178 ln x
Correlation significance	0.008	0.136	0.006

* Calculated according to method of Finney (1971).

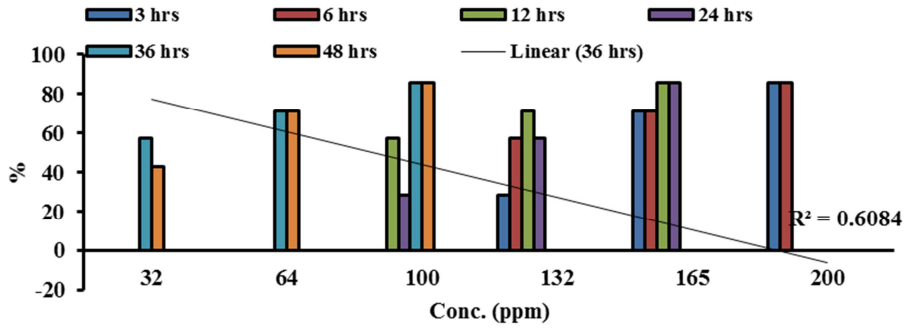

Figure 4. *Nikel effect on Lethrinus harak.*

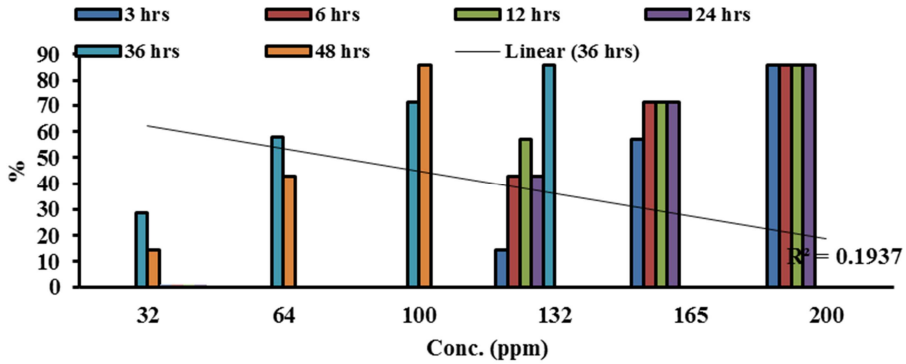

Figure 5. *Nikel effect on Cephalopholis minata.*

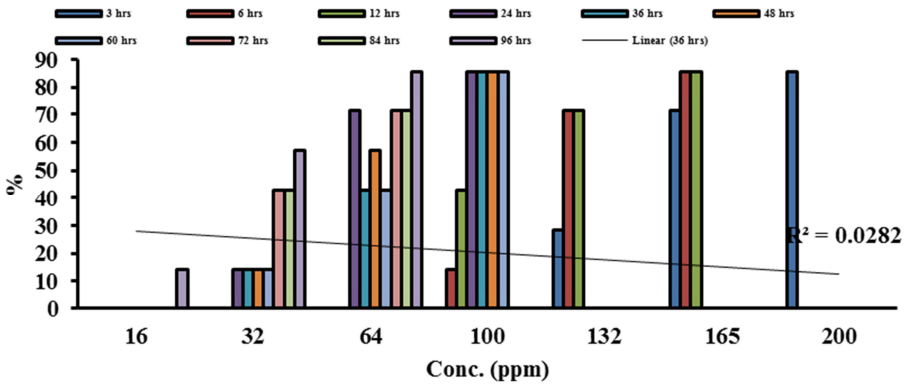

Figure 6. *Nikel effect on Tridacna maxima.*

3.3. Effects of Copper on the Mortality Rate

According to this study LT50 values of copper treatment were 35.041 hours, 47.681 hours and 71.853 hours for L. harak, C. minata and T. maxima, while that of LC50 were 197.175 ppm, 272.932 ppm and 272.841 ppm for L. harak, C. minata and T. maxima, respectively (Table 3, Figures 7, 8 and 9). The correlation coefficients between concentrations and mortality were significant ($p<0.05$) for T. maxima and C. minata, while for L. harak the correlation was highly significant ($p<0.01$).

Table 3. *Probit analysis of fishes, and mollusc, correlation between concentration (Y) and mortality (X).*

Probit analysis*	Tridacna maxima	Lethrinus harak	Cephalopholis minata
LC50	272.841	197.175	15.26557
LT50	71.853	35.041	272.932
MCR	17.66	21.90	47.681
Correlation coefficient (r)	0.74	0.80	0.85
Regression equation Y=a+ b In X	Y = 4.4244 -0.081 In x	Y = 4.7144+0.169 In x	Y = 4.6156+0.3247 In x
Correlation significance	0.012	0.003	0.012

* Calculated according to method of Finney (1971)

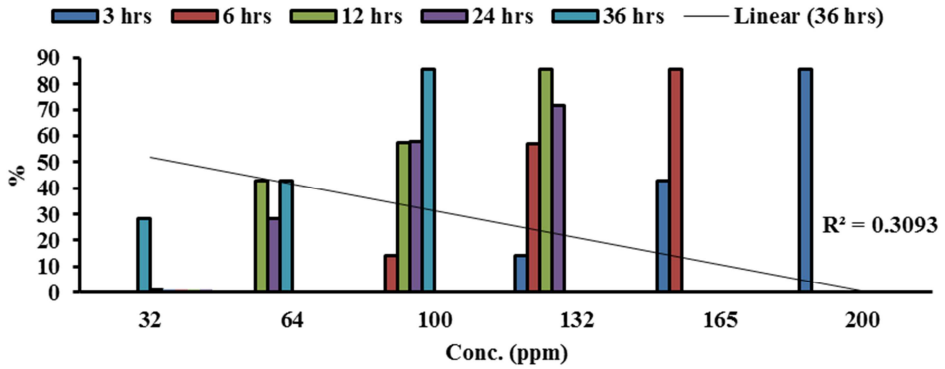

Figure 7. *Copper effect on Lethrinus harak.*

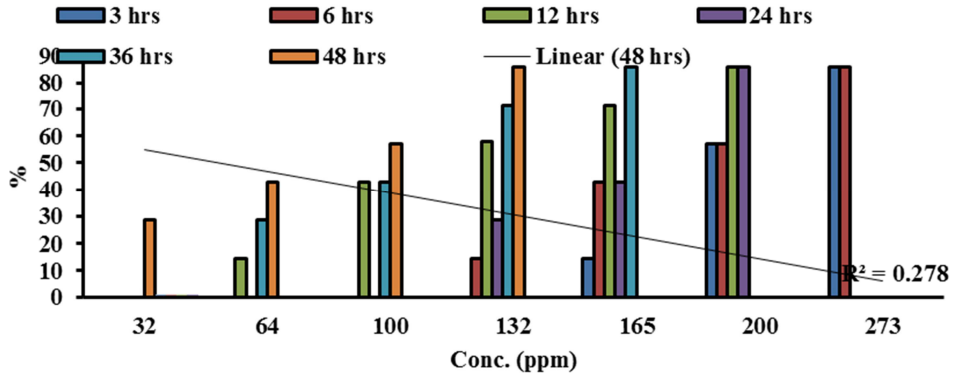

Figure 8. *Copper effect on Cephalopholis minata.*

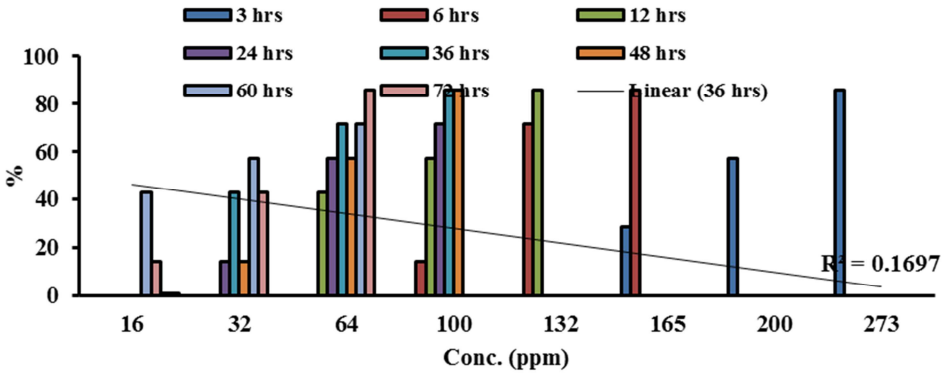

Figure 9. *Copper effect on Tridacna maxima.*

3.4. Levels of Heavy Metals in Tissues of Fishes, and Mollusc

Tables (4, 5 and 6) showed the changes in levels of the studied pollutants in tissues of the experimental animals. It is apparent from Table 4 that, the mean concentration of vanadium was below detection limit in the control. However, the mean concentration between the experimental species was significantly different as indicated by Duncan's multiple range tests.

Table 4. *Vanadium concentration in tissues of fishes, and mollusc.*

Species	Control*	Mean of vanadium
Lethrinus harak	Below detection limit.	0.010c±0.00
Cephalopholis minata	Below detection limit.	0.014b±0.01
Tridacna maxima	Below detection limit.	0.042a±0.00
P-value	0.0371	

Means bearing different superscripts are significantly different (p≤0.05) according to Duncan's multiple range tests.

It is apparent from Table 5 that, the difference of nickel concentration was highly significant in the experimental samples compared with the control. A significant difference was observed also between the experimental samples as indicated by Duncan's multiple range tests.

Table 5. *Nickel concentration in tissues of fishes, and mollusc.*

Species	Control	Mean of nickel
Lethrinus harak	<0.10d±0.00	0.561b±0.04
Cephalopholis minata	<0.10d±0.00	0.421c±0.02
Tridacna maxima	<0.10d±0.00	0.871a±0.03
P-value	0.0025	

Means bearing different superscripts are significantly different (P≤0.05) according to Duncan's multiple range tests.

Results obtained from Table 6 showed that, the difference of copper concentration was highly significant between the experimental samples and the control. Significant difference was also observed between the experimental samples as indicated by Duncan's multiple range tests.

Table 6. *Copper concentration in tissues of fishes, and mollusk.*

Species	Control	Mean of copper
Lethrinus harak	0.1652d±0.08	1.1030b±0.09
Cephalopholis minata	0.2820cd±0.07	0.4060c±0.02
Tridacna maxima	0.0624d±0.01	1.3450a±0.08
P-value	0.0	

Means bearing different superscripts are significantly different (P≤0.05) according to Duncan's multiple range tests.

With respect to L. harak the mean concentration (ppm) of copper was the highest (1.1030b±0.09), followed by nickel (0.561b±0.04) and the lowest was 0.010c±0.00 for vanadium. However the mean concentration (ppm) of nickel in C. minata was the highest (0.421c±0.02), followed by copper (0.406 c±0.02) and the lowest was vanadium (0.014b±0.01) while T. maxima exhibited the highest mean concentration (ppm) of copper (1.3450a±0.08), followed by nickel (0.561b±0.04) and the lowest was 0.042a±0.00 for vanadium.

This study throws some light on the toxic effects of heavy metals (vanadium, nickel and copper) on fishes and mollusc. The results obtained in this study indicated that the lethal concentrations LC50 of nickel were 199.236 ppm 196.041 ppm and 198.200 ppm for L. harak, C. minata and T. maxima, respectively. Copper recorded 197.175 ppm, 272.932 ppm and 272.841 ppm for L. harak, C. minata and T. maxima, respectively. However, vanadium recorded lower lethal concentrations compared to nickel and copper, these were 131.836 ppm, 164.769 ppm and 164.037 ppm for L. harak, C. minata and T. maxima, respectively, these were agreed with who worked on grass carp (Ctenopharyngodon idella) and silver carp (Hypophthalmichthys molitrix) [13]. It is also in accord with the findings of who studied nickel and copper accumulation in the liver of a freshwater fish Tor putitora. Due to exposure of nickel LT50 were 47.815 hours, 47.963 hours and 95.216 hours for L. harak, C. minata and T. maxima, respectively, and that due to exposure of copper were 35.041 hours, 47.681 hours and 71.853 hours for L. harak, C. minata and T. maxima respectively. However, vanadium recorded 11.989 hours, 5.792 hours and 47.511 hours for L. harak, C. minata and T. maxima respectively. These results confirmed that mollusc T. maxima were more tolerant than the two fish species [14].

Values of heavy metals concentrations recorded in tissues of the experimental samples were 0.561 – 0.04 ppm, 0.421 – 0.02 ppm and 0.871 – 0.03 ppm for Nickel in L. harak, C. minata and T. maxima, respectively. However, vanadium recorded 0.010 – 0.00 ppm, 0.014 – 0.01 ppm and 0.042 – 0.00 ppm for L. harak, C. minata and T. maxima, respectively. These results were agreed with who worked on bioaccumulation of nickel and vanadium in tissues (gills, liver, intestine and kidney) of the catfish Clarias batrachus, however, results related to copper concentrations in Oreochromis mossambicus were in close agreement with the present findings, similarly T. maxima results obtained were in line with those obtained by [15-17].

4. Conclusion

In conclusion, the pollutants studied were found to be highly poisoning and lethal for the experimented animals. In this experiment no response was detected in lower concentrations of nickel and copper i.e. 4 ppm and 32 ppm, while a highly response of the pollutant vanadium was detected in the same concentrations. For the three populations highest LT50 occurred at a concentration of 64 ppm of nickel and copper with significant variation in response between fish species and mollusc ($p<0.05$), and the highest LT50 of vanadium occurred at concentration of 4 ppm with significant variation in response between fish species and mollusc ($p<0.05$). For copper, variations between the two fish species were not significant ($p>0.05$), however the variation between fish species and mollusc was highly significant ($p<0.05$).

Although high concentration of nickel and copper were found in tissues of the experimented animals compared to vanadium, never the less, the results indicated that vanadium was highly poisoning than nickel & copper.

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