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A STUDY OF THE ACUTE TOXICITY OF SOME SELECTED HEAVY METALS AND PESTICIDES ON A FRESH WATER SNAIL (*Melanoides tuberculatus*).

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Abstract:
Acute toxicity of four heavy metals and two pesticides on a fresh water snail, *Melanoides tuberculatus* (Gastropod, Thiaridae) *was* investigated. Adult *M tuberculatus* was exposed for a four-day period in laboratory conditions to a range of heavy metal: Arsenic (As), Copper (Cu), Cadmium (Cd), Lead (Pb) and pesticides: Cypermethrin and Chlopyrifos. The median lethal concentration, LC_{50} values for the 96-hour exposure to As, Cu, Cd and Pb were 6.24, 8.60, 15.22 and 1082.10mg/l respectively, while the LC_{50} values for the 96-hour exposures to Chlopyrifos and Cypermethrin were 2.45and 3.81ml/l respectively. Arsenic was the most toxic heavy metal to *M. tuberculatus*, followed by Cu, Cd and Pb (As>Cu>Cd>Pb).
Between the two pesticides under study, chlopyrifos was found more toxic than cypermethrin. Mortality was assessed and median lethal concentration (LC_{50}) was calculated. Mortality increased with the increase in mean exposure concentration and times for all metals and pesticides. No mortality was recorded for the control as all the values obtained were zero.
This study indicates that *M tuberculatus* is sensitive to heavy metals and pesticides and therefore can be used to monitor the presence of pollutants at low concentration levels in waterbody.

Key words: *Melanoides tuberculatus,* LC_{50}, Heavy metals, Pesticides, Mean lethal Concentration, Mean lethal Time

INTRODUCTION

Water pollution is one the biggest issues created as a result of urbanization, industrialization and modern agricultural practices. It leads to alteration in physical, chemical and biochemical properties of water bodies as well as the environment [1].
Streams and rivers flowing through agricultural areas where pesticides and fungicides may have been applied get polluted and pose problems to the ecosystem. In addition, industrial areas where there may be deposit of metal waste present various problems when they are channeled into water bodies. Effluents discharged into river may affect aquatic animals such as fishes, snails either directly or indirectly [2].
Over the past few decades, heavy metals contamination of aquatic system has attracted the attention of several investigators [3]. The fact that heavy metals cannot be destroyed through

biological degradation and have the ability to accumulate in the environment make these toxicants deleterious to aquatic organisms and consequently to humans who depend on aquatic products as sources of food. Heavy metals can accumulate in the tissues of aquatic animals such that tissue concentrations of heavy metals is of public health concern to both animals and human [4,5].

Heavy metals are metals that are at least five times denser than water. As such, they are stable and bio-accumulative. They are sometimes passed on the food chain to humans [6]. These metals include mercury, nickel, lead, arsenic, cadmium, aluminium, platinum and copper (metallic form and ionic form). Most heavy metals have no beneficial functions to the body and can be highly toxic [6]. They are taken into the body through inhalation, ingestion and skin absorption. If they enter body tissues and accumulate faster than the body's detoxification process, then a gradual buildup of these toxic metals can occur [7,8].

Pesticides occupy a unique position among many chemicals which are encountered daily by man. They are deliberately added to the environment for the purpose of controlling pests. Indiscriminate use of different pesticides in agriculture has increased over the years, especially in the developing countries [9]. In recent years, synthetic pyrethroids have been developed for major uses in agriculture and public health purposes. The current commercial products were evolved from the natural pyrethrins, which possess high insecticidal potency, low mammalian toxicity and very short persistence. The low toxicity of synthetic pyrethroid has encouraged their uses in intensive agriculture. In agriculture, they have been used as replacements for more toxic pesticides such as organophosphates. However, some reports have indicated that non-target organisms such as aquatic invertebrates and fish are extremely sensitive to the neurotoxic effects of these pesticides when they enter surface water-courses [10, 11, 12, 13]. According to [14], cypermethrin and chlopyrifos constitute about 75 per cent of the pesticides used in most farms in the Niger Delta region of Nigeria.

Snails and slugs are among the most important terrestrial bioindicators of metal pollution because they are able to accumulate large quantities of metals in their tissues [15, 16, 17]. Using snails in toxicity bioassays is an attractive method since they are easy to culture in the laboratory, and can be fed on artificial diets with the desired amounts of metals, and respond quickly to metal contamination in the range of sublethal doses [18]. Freshwater snails have also been used to study Cadmium accumulation in their tissues [19, 20]. Gastropod mollusks are known to concentrate metals better than many of other invertebrates (21).

M. tuberculata is a specie of freshwater snail with an operculum, a parthenogenetic, aquatic gastropod mollusc in the family Thiaridae. The average shell length is about 20–27 mm and this species is native to subtropical and tropical northern Africa and southern Asia (Indo-Pacific region, Southern Asia, Arabia, and northern

Australia), but they have established populations worldwide. The snail has an operculum that can protect it from desiccation and can remain viable for days on dry land. It is a warm-climate specie which prefers a temperature range of 18-32^{0}C, and is primarily a burrowing species that tends to be more active at night. This snail feeds primarily on algae (microalgae) and acts as an intermediate host for many digenetic trematodes [22].

In Nigeria, the presence of *M tuberculatus* has been reported by a few authors. [23] examined the distribution and the habitat preference of the species in southwestern part of the country while [24] reported its occurrence also in Omi Stream, Ago-Iwoye in the southwestern part of the country.

Little information exists in the literatures concerning the toxicity of heavy metals and agricultural pesticides for this snail. So far, only few studies have been reported on metal toxicity [25, 26, 27] and most of these studies were on accumulation of metals [28, 29, 20]. It is important to conduct studies with local organisms that can be used to acquire data on pesticides and metal toxicity and to determine the organism's sensitivity to the pollutants.

Therefore, the aim of this study was to compare the acute toxicity effects of four heavy metals, Arsenic, Copper, Cadmium, lead and two selected pesticides, Cypermethrin and Chlopyrifos on a fresh water snail, *Melanoides tuberculatus.*

Materials and Methods

The Snails, *Melanoides tuberculatus* were collected from a stream draining the Awba dam in the University of Ibadan, Oyo state, Nigeria. The stream is located at the south-western part of the university. It lies between latitudes 7^0 26^1 to 7^0 28^1 North and longitudes 3^0 35^1 to 3^0 54^1 East at an altitude of 209 meters above sea level. A total of 1050 snails, *M tuberculatus* were collected by hand picking into a plastic container and transported to animal house of the department.

The shell length was measured with the use of a divider and a meter ruler measuring from the apex to the topmost edge of the aperture. Their weight was also taken.

Prior to toxicity testing, the snails were acclimatized for two weeks in 5 litres stocking glass aquaria using dechlorinated tap water. During acclimatization period, the snails were fed with dried blanched pawpaw leaves.

HEAVY METALS BIOASSAY PROCEDURE

A range finding test was conducted for 96-hour period to determine the concentrations of the heavy metals to be used in the actual experiment using standard procedure following the methods of American Public Health Association [30].

The standard stock solution (100mg/l) of Cu, Cd, Pb and Ar were prepared from analytical grade metallic salts of $CuSO_4.5H_20$, $CdSO_4$, $Pb(NO_3)^2$, and $Ar(NO_3)^2$ respectively. The stock solutions were prepared with deionized water in 1 litre volumetric flask. Acute toxicity experiment of Cu, Cd, Pb and Ar were performed for a four-day period using adult snails (shell length approximately (1.6-2.2cm, mean wet weight 23.5±1.8mg) obtained from stocking tanks.

Following a range finding test, the following were chosen for each of the metallic salts: $CuSO_4.5H_20$ (2, 5, 10, 15 and 20 mg), Pb $(NO_3)^2$ (100, 150, 200, 250 and 300 mg), $CdSO_4$ (5, 10, 15, 20, and 25 mg/l), and $Ar(NO_3)^2$ (2, 5, 10, 15 and 20 mg/l). Metal solutions were prepared by serial dilution of a stock solution with dechlorinated tap water. A control with dechlorinated tap water only was also used. The tests were carried out under static conditions with renewal of the solution every two days. Control and heavy metal-treated groups each consisted of three replicates of ten (10) randomly allocated snails in a 5 litres glass aquarium containing appropriate solution. No stress was observed for the snails in the solution, indicated by 100% survival for the snails in the control water until the end of the study. A total of 10 snails per treatment/concentration were used in the experiment. Mortality was observed at 24, 48, 72 and 96 hours of exposure and during observation, dead ones were removed immediately from the glass aquaria.

CYPERMETHRIN AND CHLOPYRIFOS BIOASSAY PROCEDURE

A range finding test was conducted for 96-hour period to determine the concentrations of Cypermethrin and Chlopyrifos to be used in the actual experiment using standard procedure following the methods described by [30]. Based on the results of the range finding test, five concentrations of the test solutions (2.5, 3.5, 4.5, 5.5, 6.5ml/l) for Cypermethrin and four concentrations (1.5,2.5,3.5, and 4.5ml/l) for Chlopyrifos and a control (0.0ml) were prepared by serial dilution of the stock solution according to the method described by (31) in glass aquaria in triplicates.

Acute toxicity experiments were performed for a four-day period using adult snail (shell length approximately 1.6-2.2cm; mean wet weight (23.5±1.8mg) obtained from stocking glass aquaria. Control and chemical treated groups contained ten (10) randomly allocated snails in 5 litre glass aquaria.

QUANTAL RESPONSE

In this study, the specimens were considered dead when they failed to retract their propodial foot into their shell upon prodding with glass rod or failure to protrude out of its shell during the period of observation. Mortality was monitored and recorded every 3 to 4 hours for the first two days and then at 12 to 24 hour intervals throughout the rest of the period.

CONTROL TEST

Control is an integral part of toxicity test and was done to ascertain if the death of organisms were due to toxicant or some other factors. Control tests were typically conducted by placing the organisms in dechlorinated water with no toxicant. As a rule, a toxicity test is valid if control mortality was less than 10% according to [32].

PHYSICO-CHEMICAL PARAMETERS

Physico-chemical parameters (Temperature, pH, Conductivity, salinity and Dissolved Oxygen) were measured every day.

TEMPERATURE

Water temperature was measured to the nearest $0.1^{0}C$ with a mercury-in-glass Thermometer of range $10\text{-}110^{0}$ which was inserted at a depth of about 2cm from surface for three minutes. The sub-surface temperature was taken immediately after collection with Kemmer water sampler. The temperature was taken three times and the average was calculated to be the average temperature. The readings were expressed in degrees Celsius (^{0}C).

pH

The pH was measured electrically with an electronic pH metre, Kent 7020 model. The glass probe of the meter was dipped into the water sample and the pH read at room temperature as recommended by [30].

CONDUCTIVITY

Conductivity was determined in the laboratory using conductivity and TDS combined meter Matini instruments model. Some quantity of water was poured into a small beaker and the probe was put into the beaker and measured. The results were expressed in μS/cm.

SALINITY

Salinity was determined with "HACH Sach's fish farmer's water quality test kit" (HACH company, USA).

DISSOLVED OXYGEN

Dissolved Oxygen was determined using Winkler's method as described by [30].

STATISTICAL TESTS

The 24, 48, 72 and 96 hours LC_{50} values, 95% confidence interval values, Chi-Square values, slopes and intercepts were obtained using Probit analysis computer programme.

Data obtained from the physiochemical parameters were analyzed by a one-way analysis of variance (ANOVA) test at 0.05% probability level.

RESULTS AND DISCUSSION

The LC_{50} values of the heavy metals on *M tuberculatus* are presented in Table 1, 2, 3, 4, while tables 5 and 6 show the LC_{50} values of the pesticides on the snails. The Probit line graphs of the toxicity data for *M tuberculatus* exposed to different acute concentration of heavy metals and pesticides are also presented in Fig 1, 2, 3, 4 and 5. The percentage mortality of *M tuberculatus* exposed to acute concentration of Cypermethrin and Chlopyrifos and four heavy metals was concentration dependent.

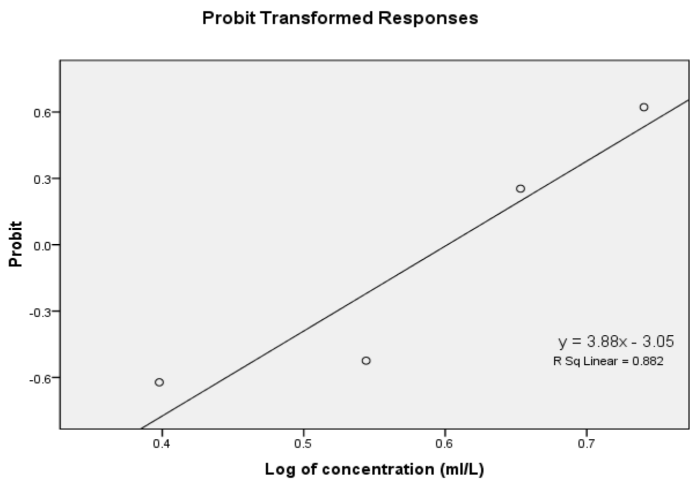

Figure 1: Probit line graph of acute toxicity Cypermethrin to *M tuberculatus*

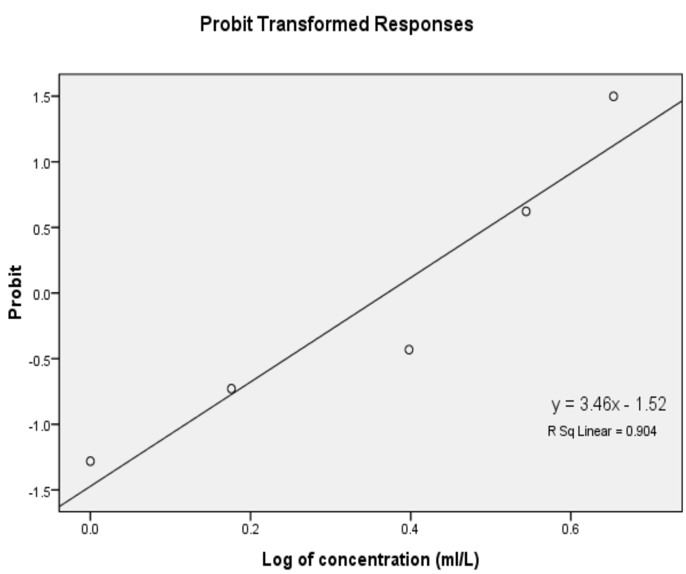

Figure 2: Probit line graph of acute toxicity Chlopyrifos to *M tuberculatus*

Figure 3: Probit line graph of acute toxicity of Copper sulphate to *M tuberculatus*

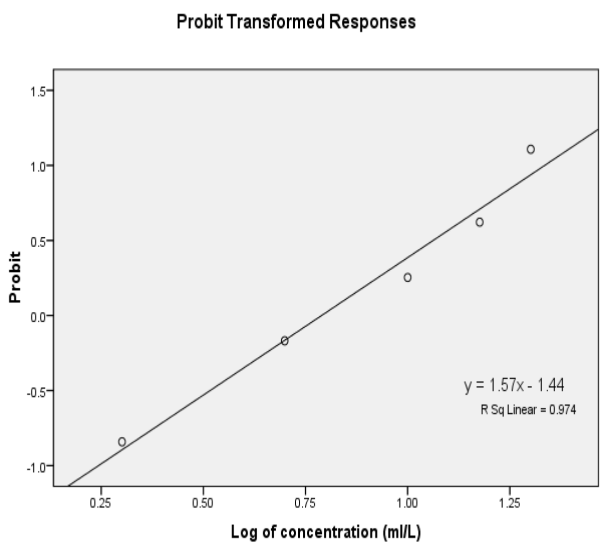

Figure 4: Probit line graph of acute toxicity of Arsenic nitrate to *M tuberculatus*

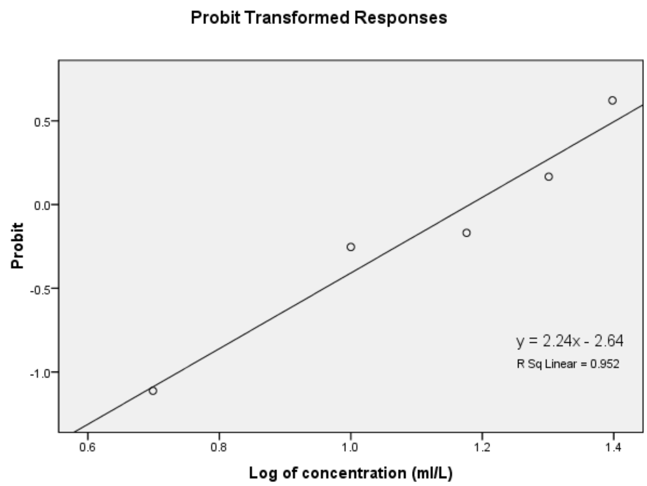

Figure 5: Probit line graph of acute toxicity of Cadmium sulphate to *M tuberculatus*

The 24hr, 48hr, 72hr and 96hr median lethal concentration (LC_{50}) of four (4) selected metals: Ar, Cu, Cd and Pb with two (2) selected pesticides: Chlopyrifos and Cypermethrin and their 95% confidence limits are presented in Tables 1, 2,3,4,5 and 6. The 96hr-LC_{50} values of the four heavy metals (Cu, As, Cd and Pb) under study was 8.60, 6.24, 15.23 and 1082.10 mg/L respectively. The 96hr - LC_{50} of Cypermethrin in this study was found to be 3.81ml/L while that of Chlopyrifos was 2.45ml/L. Comparison of the acute toxicity effects of the four metals on *M tuberculatus* showed that Arsenic was more toxic (6.24), followed by Copper (8.60), Cadmium (15.23) and Lead (1082.10). The 96hr - LC_{50} values obtained from acute toxicity of the pesticides showed that Chlopyrifos (2.45) was more toxic than Cypermethrin (3.81). Mortality of the snails increased with increased time and concentrations. There was no mortality observed in the control set up.

Table 1: LC_{50} values of Copper sulphate to fresh water Snail *M tuberculatus.*

Copper sulphate

Time (hr)	LC_{50} mg/l	95% confidence limits Lower	Upper
24	34.09	25.95	56.28
48	16.71	14.89	24.62
72	11.09	6.15	24.62
96	8.60	3.09	20.49

Table 2: LC_{50} values of Arsenic nitrate to fresh water Snail *M tuberculatus.*

Arsenic nitrate

Time (hr)	LC_{50} mg/l	95% confidence limits Lower	Upper
24	98.44	-	-
48	48.96	31.91	199.39
72	26.92	20.01	43.80
96	6.24	5.26	43.80

Table 3: LC_{50} values of Cadmium sulphate to fresh water Snail *M tuberculatus.*

Cadmium

Time (hr)	LC_{50} mg/l	95% confidence limits Lower	Upper
24	30.26	-	-
48	49.19	-	-
72	24.97	15.86	36.92
96	15.23	13.50	17.33

Table 4: LC_{50} values of Lead nitrate to fresh water Snail *M tuberculatus*

Lead

Time (hr)	LC_{50} mg/l	95% confidence limits Lower	Upper
24	717.67	450.80	3508.43
48	1539.98	-	-
72	663.53	-	-
96	1082.10	572.85	22552.5

Table 5: LC_{50} values of Cypermethrin to fresh to fresh
water Snail *M tuberculatus.*

Cypermethrine

Time (hr)	LC_{50} mg/l	95% confidence limits Lower	Upper
24	6.51	5.06	25.63
48	4.89	4.55	5.32
72	4.29	2.91	5.92
96	3.81	1.59	5.59

Table 6: LC_{50} values of Chlopyrifos

water Snail *M tuberculatus*

Chlopyrifos

Time (hr)	LC_{50} mg/l	95% confidence limits Lower	Upper
24	5.51	4.65	7.14
48	4.91	2.20	15.11
72	2.50	1.81	5.04
96	2.45	2.45	3.86

The results of the physiochemical parameters of the sample units and the control are presented in the Table 7. The temperature, pH, Conductivity, TDS, salinity, Dissolved Oxygen (DO) and BOD (Biochemical Oxygen Demand) are: 29.34±1.56^{0}C, 7.86±1.34, 384±34.2 (µs/cm), 234±18.41 (mg/l), 0.21±0.20, 0.23±0.12 (mg/l) and 0.27±0.20 respectively. There was no significant difference (P≤0.05) between the values obtained from the test media and the control.

Table 7: Physicochemical parameters of the experimental and control units

Parameters	Experimental Units			Control Units		
	Min	Max	Mean±SE	Min	Max	Mean±SE
Temperature (^{0}C)	28.25	30.42	29.34±1.56	27.98	31.00	29.49±1.45
pH	7.56	8.02	7.86±1.34	7.55	7.98	7.77±1.20
Water hardness (mg/l)	20.24	28.68	24.26±2.35	18.60	19.00	18.80±1.89
Conductivity (µS/cm)	376.6	492.2	384±34.20	374.2	471.5	422.9±32.89
TDS (mg/l)	200	262.5	234±18.41	200	270.0	235±16.22
Salinity (‰)	0.20	0.22	0.21±0.20	0.20	0.21	0.21±0.20
DO	0.16	0.46	0.23±0.12	0.20	0.52	0.36±0.10

Table 8: Comparison of LC_{50} values of Melanoides with other fresh water molluscs

Heavy metals	Species	Live stage	Test duration	LC_{50}	Reference
1. Copper	*M. tuberculata*	Adult	96 h	8.60	This study
	M. tuberculata	-	48 h	3.6	[26]
	M. tuberculata	Juvenile	24 h	0.2	[25]
	B. glabrata	Adult	48 h	0.18	[33]
	P. paludosa	60 d	96 h	0.14	[34]
	P. jenkinsi	Adult	96 h	0.08	[35]
2. Cadmium					
	M. tuberculate	Adult	96 h	15.22	This study
	P. fontinalis	—	96 h	0.08	[36]
	V. bengalensis	—	96 h	1.2	[37]
	L. luteola	Adult	96 h	1.5	[38]
3. Lead					
	M. tuberculata	Adult	96 h	1082.10	This study
	Filopaludina sp.	Adult	96 h	190	[39]
	V. bengalensis	—	96 h	2.54	[40]
	A. hypnorum	—	96 h	1.34	[41]
4. Arsenic					
	M tuberculatus	Adult	96h	6.24	This study
	Anabas testudineus	Adult	96h	18.21	[42]
5. Cypermethrin					
	M tuberculatus	Adult	96h	3.81	This study
	Brycon amazonicus	Adult	96hrs	30.0	[43]
	Heteropneustes fossilis	Adult	72hr	0.67	[44]
6. **Chlopyrifos**					
	M tuberculatus	Adult	96hr	2.45	This study
	Cambarellus montezumae	Adult	72hr	5.20	[45]

This present study indicated that Arsenic was highly toxic to *M tuberculatus* while lead has the least toxicity effect on the animal. The order of increasing toxicity of metals to *M tuberculatus* was As>Cu> Cd >Pb. Similar results were obtained by [27], they found that Cu was the most toxic metal to *M. tuberculatus*, followed by Cd, Zn, Pb, Ni, Fe, Mn, and Al (decreasing order). [46] also obtained similar results for marine mollusks *C cingulata* and *M philippinarum*, where copper was found to be the most toxic metal and Lead was the least toxic metal. [25] and [26] showed that 96 h-LC_{50} of Cu to *M. tuberculatus* were 0.2 and 3.6 mg/l, respectively, which were lower than the present study. The difference between the values of Cu obtained from this study and that obtained by [25] and [26] may be due to differences in the characteristics (pH, Conductivity, Total

dissolved Solid, and Temperature) of the test medium. The toxicity reported by other researchers
(Table 8) differs from this present study due to the different species, ages, and sizes.

According to [47] the rank order of toxicity of metals will vary between organisms. With *Lymnaea luteola*, [48] showed that the order of toxicity was Cd > Ni > Zn; with *Viviparus bengalensis*, [37] and [40] found that the order of toxicity was Zn > Cd > Pb > Ni; and with *Juga plicifera*, [49] found that Cu was more toxic than Ni. [50] reported that the snail has a sealing operculum that allows it to withstand desiccation as well as increasing its tolerance for chemicals.

The present investigation reveals that mortality increased as period of exposure increased and rate of mortality increased as concentration of heavy metals increased. The results indicate that the effect of heavy metal was dose dependent. This agrees with the results obtained by [29].

The 96hr - LC_{50} toxicity values for the pesticides showed that Chlopyrifos (2.449 mg/l) is more toxic than Cypermethrin (3.810mg/l). Chlopyrifos is a broad spectrum organophosphate pesticide that acts by interfering with the activities of cholinesterase, an enzyme that is essential for the proper working of the nervous system. It is observed that the snails showed physiological response to the pesticide, chlopyrifos during exposure. Few minutes after exposure, some snails protruded out their propodial foot from their shell; secreted mucus, wanted to move out of water while some retracted totally into the shell. It was also observed that some snails emptied the content of their gastro-intestinal tracts and retracted into the shell when exposed to cypermethrin. These were a behavioural or physiological response to reduce the rate of metabolism and not allow entry of any materials into the body.

From this study, it was observed that mortality increased as period of exposure increased and rate of mortality increased as concentration of Chlopyrifos and Cypermethrin increased.

Cypermethrin and Chlopyrifos have been observed to be toxic to other aquatic organisms such as Mud crab *Callinectes sapidus* and blue crab *Rhithropanopeus harisii*) [51]; Crayfish, [52]; Tilapia [53] and mud periwinkle and shrimp, [54].

There was no significance difference ($P \leq 0.05$) between the values of the physiological parameters of the experimental media and the control. Also, no mortality was recorded in the control media throughout the period of this study. Therefore mortality of *M tuberculatus* was due to exposure to heavy metals and pesticides.

Despite the important role played by pesticides in the control of pests to enhance agricultural yields, indiscriminate applications may pose great threats to non-target organisms. Results from this study strongly suggest that Arsenic, copper, Cadmium, lead, Cypermethrin and Chlopyrifos could be toxic to *M. tuberculatus* if they find their way into the aquatic environment even at very low concentration and affect aquatic biota.

The determination of LC_{50} value is highly useful in the evaluation of safe level or tolerance of pollutant and it provides fundamental data for the design of more complex disposal models.

CONCLUSION

This study showed that heavy metals such as copper, arsenic, cadmium and lead and also pesticides like Cypermethrin and Chlopyrifos were very toxic to *M tuberculatus*, and that the snail was sensitive to metals. It should be noted that accidental discharge of heavy metals from industrial effluents and pesticides used on farms to control pests could be very disastrous to non-target organisms like snails, macro-invertebrates and fish.

The present acute toxicity test using *M tuberculatus* could be an important component of the routine battery of tests to evaluate toxicity of heavy metals, support registration of pesticide products for outdoor application, monitor effluents, establish water quality criteria and provide aquatic safety assessments for chemicals.

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