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Acute Toxicity of Cadmium (Cd) and Mercuric (Hg) on freshwater mussel *Lamellidens marginalis* (Lamarck, 1819) from Godavari River in Maharashtra, India

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Abstract: *The lives of fresh water bivalve mussels are sensitive to bio-accumulation of heavy metals due to its bio-filtering nature. We measured acute toxicity and sublethal effect of Cadmium (Cd) and Mercuric (Hg) metal on freshwater bivalve mussel Lamellidens marginalis collected from Pravara-Sangam of Godavari River in Maharashtra. Adult animals L. marginalis (65-70 mm in length size) were exposed for 4 days period in laboratory conditions where mortality was calculated and the LC_{50} values for the 96-hour exposures to Cd and Hg were 12 and 6 ppm respectively. Metal bio-concentration in freshwater bivalve mussel is increased with exposure to their increasing concentrations. Maximum bio-accumulation of metal observed in the soft tissue of animal and protein depletion observed in gills, foot and digestive gland (hepatopancreas) tissues were significantly. Investigation confirm higher sensitivity of freshwater bivalve mussels to mercuric (Hg) than cadmium (Cd) metal.*

Key Words: *Acute Toxicity, Metal (Cadmium and Mercury), Lamellidens marginalis.*

1. INTRODUCTION:

The rapid industrialization becomes one of the major causes of contamination in the aquatic ecosystem. Heavy metals are one of such pollutants that may come from both natural and human activities (Mountouris et al. 2002; Jagtap, 2011; Azizi, 2018; Binder et al, 2019). Industrial toxic pollutants are directly entering into the aquatic environment which cannot be degraded, therefore, it accumulates in the water, soil, sediments and living organisms that lead to contaminant the food-chain and thus it becomes a global issue (Wang et al, 2014, Dhara et al, 2017, Khan et al, 2018). The effect of these metals on aquatic life considered being a major threat because of their toxicity, long persistence, and bio-accumulation and bio-magnification in nature. Numerous heavy metals are nutrients at their trace levels while Pb, Cd and Hg are non-essential and are recognized as important industrial hazards. It has the ability to be accumulated in benthic and filter-feeding animals causing severe toxic effects upon acute exposure (Waykar and Shinde, 2011). They are bind to the cell membrane and are very difficult to remove from the body of animals and have great ecological concern worldwide. There are several studies published on the accumulation of heavy metals in aquatic life exposed to ecological pollution and interrupt their physiological processes in an increasing manner (Naimo, 1995; Abd Allah et al, 1997; Mountouris et al. 2002; Waykar and Shinde, 2011; Samecka-Cymerman and Kempers, 2007; M.B.Jorge et al. 2017; Waykar & Deshmukh, 2011; Yueh-Min Chen, 2014; Xuelu, 2014; Suryawanshi, 2017; Torres et al, 2018; Binder et al, 2019). These ecological and eco-physiological studies advocate that mussels react to biological stress and pollution by modifying behaviour, and various life-history traits in a manner consistent with maintaining population fitness. Bivalve molluscs are sensitive to change in physiology and essential organism of aquatic environment making to them an excellent environmental indicator (Rutzke et al. 2000; Markich et al, 2002; Yusof et al, 2004; Carroll et al, 2009; Shuhaimi-Othman et al, 2012; Azizi et al, 2018; Vaughn, 2018). They are filter feeder and highly tolerant to pollutants exhibit extreme levels of metals accumulations in their body. Molluscs are abundant in aquatic ecosystems and easily available for collection (Shanmugam et al, 2000; Kharat et al 2009; Raghava 2013). The Godavari is a major eastward river of the state rises from the north-western region of Western Ghats just about 80 km away from the Arabian Sea and flow most part of its course across the broad plateau of the Deccan peninsular region. The ecological status of Godavari is strongly affected by anthropogenic activities and toxic pollution might cause environmental risk for an aquatic environment. These ecosystems are gradually coming under permanent pressure of anthropogenic activity (Jagtap et al, 2011; Wang et al, 2014; Mane, 2014; Suryawanshi, 2017; MPCB, 2019). So the monitoring and continuous assessment is gaining importance. To assess the toxicity of toxicants from aquatic environment toxicity is an essential tool is been widely used to identify an appropriate organism as a bio-indicator (Shuhaimi-Othman et al, 2012; Gnatyshyna et al, 2019). A freshwater mussel Lamellidens marginalis have been selected to understand the accumulation pattern, physiological change and acute toxicity of

cadmium (Cd) and Mercury (Hg). This study will show the outline of acute toxicity and impact on protein depletion in freshwater bivalve mussels.

2. MATERIALS AND METHODS:

Freshwater bivalve mussels *Lamellidens marginalis* of about 65-70 mm in length size from Pravarasangan of Godavari River were collected and transferred to the laboratory. Animals were acclimatized in a well-maintained aquarium for a week, water changed after an interval of 11-12 hrs every day. Water samples were also collected from the same collection site and analyzed for various Physico-chemical parameters using standard methods (APHA, 2010). Animals were prepared in a group of 10 animals and kept into five cleaned aquariums filled with 20-liter water in each. Cadmium (Cd) and Mercuric (Hg) were used as toxicants and the experiments were conducted for 96 hrs. Test solutions were renewed once after 24 hrs replacing the test solution (Chavan and Mule, 2014). The first aquariums were kept as a control and the remaining four were experimental. Cadmium (Cd) and Mercuric (Hg) doses were given to the selected bivalve in 10, 11, 12, 13, and 5, 6, 7, 8 ppm respectively. Live bivalves from the LC_{50} group were anesthetized with chloroform by using the standard method (Ostaszewska et al, 2005); and then the foot, gills, and digestive glands were dissected. Samples were dried for 3-4 days to remove water content; it transferred to the hot air oven maintained at 90-100 0C. Sampled 20 mg of dried tissue of the foot, gills, and digestive gland were weighed separately and it homogenized in 1N NaOH; centrifuged at 3000 rpm for 15 min, the supernatant was discarded; 5ml of alkaline copper Sulphate reagent were added and allowed to stand at room temp for about 10 min. After that, 0.5ml of Folic-phenol reagent (1:1 folic-phenol: distilled water) added solution was mixed thoroughly and incubated at room temp for about 30 min and the measured color developed at 650 nm. Obtained values were calculated against BSA used as standard and were represented as mg protein/gm dry weight of the tissue, where whole proteins were estimated by Lowry et al, (1951).

Study area Figure1: Pravara-Sangam of Godavari River of Ahmednagar District, MS, India.

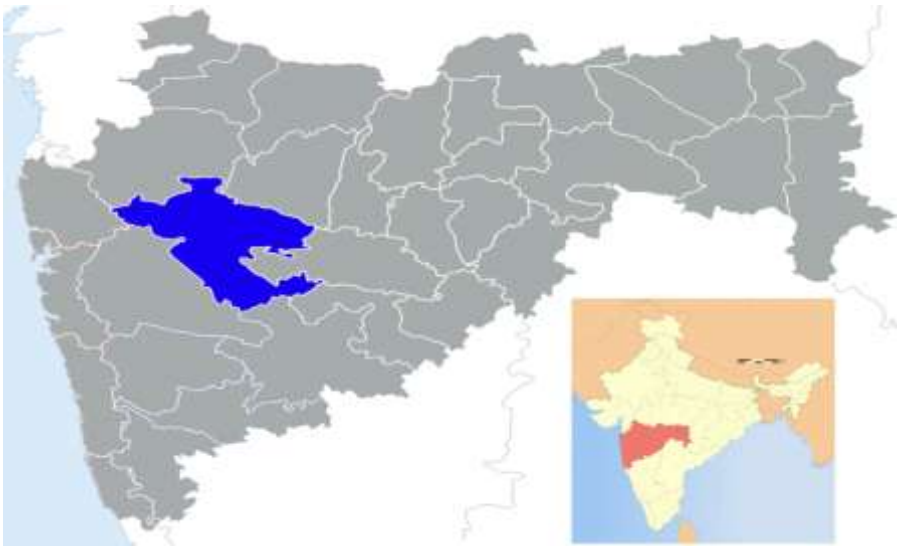

Figure 2: Map Showing Stretch of River Godavari and sample collection centres in Godavari river of Maharashtra state

3. RESULTS AND DISCUSSION:

In the first experiment, freshwater mussel *Lamellidens marginalis* were exposed to Cadmium (Cd) concentration. The experiments shows within 96 hrs, the mortality rate was recorded in 10 ppm were 30 %, in 11 ppm were 40%, while in 12 ppm were 50% and in 13 ppm were 60 % shown in the table 1 and figure 3.1.

Table 1: *L. marginalis* Percent and Probate Mortality of Cadmium (Cd)

Cadmium (Cd) (ppm)	Dead animal	Live animal	Mortality %	Probate mortality
Control	0	10	0.0	0.0
10	3	7	30	4.48
11	4	6	40	4.75
12	5	5	50	5.00
13	6	4	60	5.25

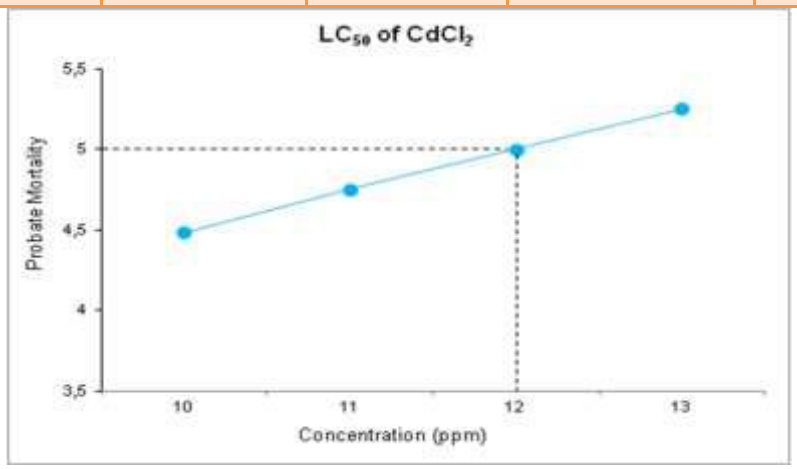

Figure 3.1: *L. marginalis* LC_{50} of Cadmium (Cd)

In second experiment freshwater mussel *Lamellidens marginalis* were exposed to the Mercury (Hg) concentration. The experiments shows within 96 hrs, the mortality rate was recorded in 5 ppm were 40%, in 6 ppm were 50%, while in 7 ppm were 60 % and in 8 ppm were 70% and shown in the table 2 and figure 3.2.

Table 2: *Lamellidens marginalis* Percent and Probate Mortality of Mercury (Hg)

Mercury (Hg) (ppm)	Dead animal	Live animal	Percent mortality %	Probate mortality
Control	0	10	0.0	0.0
5	4	6	40	4.75
6	5	5	50	5.00
7	6	4	60	5.25
8	7	3	70	5.52

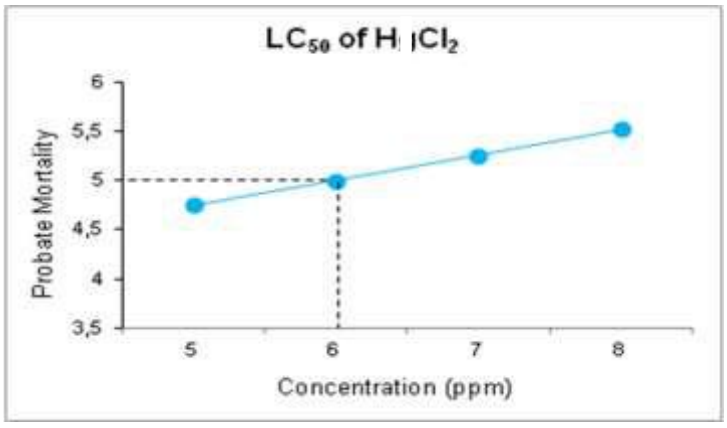

Figure 3.2: *Lamellidens marginalis* LC_{50} of Mercury (Hg)

The determinations of the LC_{50} values have immense importance since it provides primary data to design additional and multifaceted disposal model. Generally, the accumulation of heavy metal affects the behaviour of test animals and reduced their survival rate. There are a significant number of reports on the studies carried out on the effects of heavy metals on freshwater mussels (Shanmugam et al., 2000). The concept of freshwater mussels watch was proposed by Goldberg in 1975 (Khan et al., 2018) because of its accumulation behaviour and capacity for being a good environmental indicator. Acute toxicity of heavy metal content in the foot, gills, and digestive glands and mantle can be used as a sub-lethal bio-indicator to monitor the water parameter, physiology of mussel, and their immune response to toxicity (Khan et al., 2018). For several decades freshwater mussels have been used for the study of environmental monitoring and river, lakes pollution (Domouhtsidou and Dimitriadis, 2000; Gundacker, 2000; Liu et al., 2010; Suryawanshi, 2017; Azizi et al., 2018). But the accumulative index of the species cannot be a selection criterion for being a good pollution indicator. In the present study, the freshwater mussels Lamellidens marginalis have established its LC_{50} values for Cadmium (Cd) and Mercury (Hg) was 12 ppm and 6 ppm respectively (shown in Fig. 3&4). The mortality rate has drastically increased with their increasing concentration and the time of exposure particularly for Cadmium (Cd) and Mercury (Hg). Hence the mortality rate is directly proportional to the time exposure and concentration of the heavy metal accumulation. Since the LC_{50} value of Cadmium (Cd) within 96 hrs was less (12 ppm) than Mercury (Hg) (6 ppm). According to Azizi et al., (2018) mussels can accumulate certain metals to high concentrations without their adverse effects, for example, cadmium can concentrate in the kidney for detoxification (such as by chelation) without adversely affecting that organ. There is a history of short-term exposure of metals like Cu, Cd, Hg, and Zn to the potentially toxic in a number of organisms. The study clearly showed that any kind of pollutant or heavy metal present in the aquatic ecosystem is toxic. The toxic metal once gets entered in the body then they certainly damage and weaken the mechanisms concern, such damage may be at a cellular and molecular level.

Studies were also done on changes in biochemical composition in the soft tissues of freshwater mussels *Lamellidens marginalis* foot gill, and the digestive gland was exposed to acute concentrations of Cadmium (Cd) and Mercury (Hg) for 96 hrs along with control group. The obtained data was supported by various statistical analyses and processed for the standard deviation of the mean was calculated (Raghava Kumari, 2013). There is a history of a metal exposure to organism which determined the body burden influences on their physiological or behavioural response to metals (Widdows et al. 1984) Table 3 and figures 3.2 represents the depletion in the amount of total protein. When the result compared with the control group there is a significant depletion found in total protein because of Cadmium (Cd) toxicity. The significant decrease in protein level was observed in all the three organs of the set. The total amount of protein in the gill of control bivalve was observed 11.4351 mg/100mg, it was significantly reduced after 96 hrs and found 7.9064 mg/100mg (-30.8584%), while foot showed 9.3099 mg/100 mg of protein in the control group, but it was decreased up to 6.6870 mg/100 mg (-28.1732%). In the digestive gland of control bivalve showed 13.6249 mg/100mg of protein in it, but it was depleted after 96 hrs as 8.7363mg/100 (-35.8798%) exposed to lethal concentration (12 ppm) of cadmium (Cd).Table 4 and figure 4 show depletion in the amount of total protein. The protein depletion occurred due to mercuric chloride toxicity has been compared with control group of bivalves. Significant decrease in protein level was observed in all the three organs.

Table 3: Total Protein content of Gill, Foot and Digestive gland of *Lamellidens marginalis* Exposed to **Cadmium (Cd)** (Mean ± S.D; n = 3)

Tissue	96 hr Control	96 hr (12ppm) Experiments	%Variation
Gill	11.4351 ±0.8086	7.9064 ±0.2701	30.8584
Foot	9.3099 ±0.6862	6.6870 ±0.7532	28.1732
Digestive Gland	13.6249 ±0.5908	8.7363 ±0.5414	35.8798

(Values expressed as mg/100mg of Dry wt. of tissue.± indicates S.D. of 3 observations.)

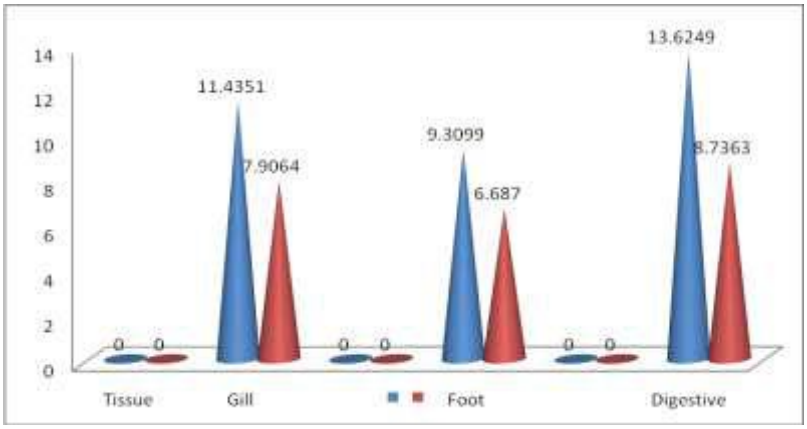

Figure 3.3: Graphical representation of Protein Alteration in the Gill, Foot and Digestive Gland of *Lamellidens marginalis* exposed to LC_{50} Cd (Mean ± S.D; n = 3)

The total amount of protein in the Gill of control bivalve was observed 11.4351 mg/100mg but it was significantly reduced after 96 hrs as 7.110 mg/100mg (-37.8195%), Foot showed 9.3099 mg/100 mg of protein in control group, but it was decreased up to 6.7167 mg/100 mg (-27.8542%), Digestive gland of controlled bivalve showed 13.6249 mg/100mg of protein in it, but it was depleted after 96 hrs as 8.2458 mg/100 (-39.4799%) exposed to lethal concentration (6 ppm) of mercury.

Table 3.4: Total Protein content in the Gill, Foot and Digestive gland of *Lamellidens marginalis* exposed to **Mercury (Hg)** (Mean ± S.D; n = 3)

Tissue	**96 hr Control**	**96 hr (6ppm) Experiments**	**% Variation**
Gill	11.4351 ±0.8086	7.1104 ±0.5540	37.8195
Foot	9.3099 ±0.6862	6.7167 ±0.6319	27.8542
Digestive Gland	13.6249 ±0.5908	8.2458 ± 0.8729	39.4799

(Values expressed as mg/100mg of Dry wt. of tissue. ± indicates S.D. of 3 observations.)

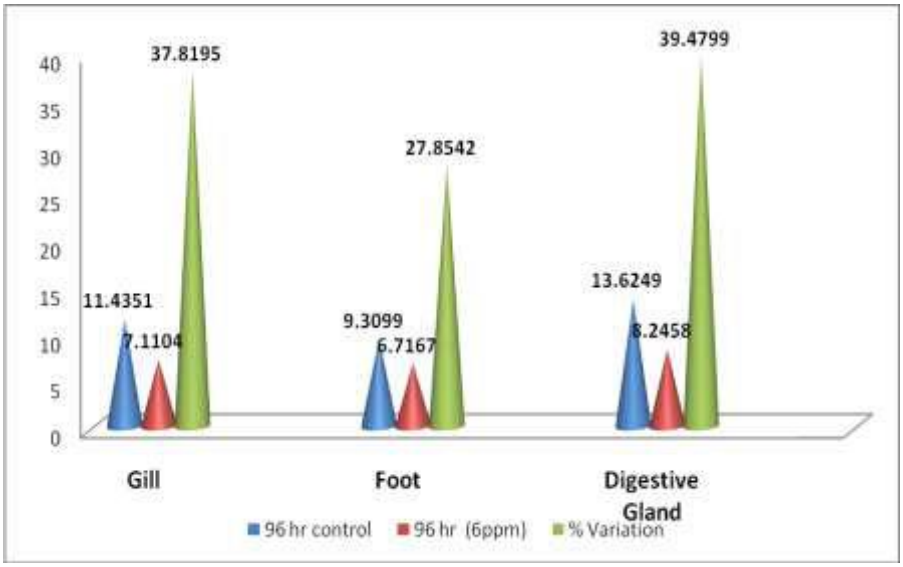

Figure 3.4: Graphical representation of Protein Alteration in the Gill, Foot and Digestive Gland of *Lamellidens marginalis* exposed to LC_{50} Hg (Mean ± S.D; n = 3)

There are several possibilities of the effect of the acute toxicity of metals in the freshwater bivalve molluscs are discussed by Suryawanshi (2017), the ability of various toxic metals to form complexity in the physiological processes

of an organism. Molluscs have the general tendency of accumulation in different tissues and not to be excreted out of the body. It is an important trend of assessment the changes in the availability of metals in the body parts of freshwater bivalve mussels. It has also recorded that molluscs can take metal from the ambient water and inorganic particulates and seasonal variation may found in metal accessibility in such mussels may have a composite function of these factors (Sadiq, 1992; Suryawanshi, 2017). In any stressful condition that alters the biochemical composition in aquatic animals. So the protein depletion may be due to increased catabolism and decreased anabolism of proteins during pollutant exposure. The trend has been recorded by various researchers (Singaraju et al., 1991; Sole et al., 2000; Kharat et al., 2009; Pourang et al., 2010; Rane and Mahajan, 2012; Raghava Kumari, 2013; Suryawanshi, 2017) of total protein depletion in different tissues of bivalve molluscs after exposure to metal pollutant and other heavy metals. It is also reported that the reduction in total protein content in the gill, foot, hepatopancreas and muscles of the *Macrobrachium kistnensis* exposed to different concentrations by Kharat et al., (2009) of tributyltin chloride, and it was possibly due to stress condition caused by toxicity on protein metabolism similar results were reported by Sole et al. (2000) and Suryawanshi, (2017). The significant decrease in protein level was observed in foot, gills and digestive glands. Freshwater mussels *Lamellidens marginalis* exposed to the lethal concentration of cadmium (12ppm), the amount of total protein in Gill, Foot and Digestive gland was -30.8584 %, -28.1732% and -35.8798%) respectively while exposed to mercuric chloride (6ppm) in Gill, Foot and the digestive gland was -37.8195%, -27.8542% and -39.4799% respectively were found significantly lower compared to control group.

There is higher protein depletion in digestive glands followed by gills and foots. The depletion might be due to more utilization of these constituents under the stress of Cadmium (Cd) and Mercury (Hg) contamination. However, the present study clearly indicated that digestive gland was the most affected tissue followed by gill and foot of Lamellidens marginalis. The higher depletion might be due to high metabolic potency and efficiency of the gland under contaminant stress and are provided a better indication of the extent of toxicity. The digestive gland seems to be the main site of degradation and detoxification of toxicants and hence has the largest demand for energy for the metabolic processes resulting in increased utilization of protein to meet energy demand. Protein depletion observed more in digestive gland exposed to Mercury (Hg) than the Cadmium (Cd), Hence the investigation also confirmed that the species has found higher sensitivity to Mercury (Hg) compared to Cadmium (Cd). There are several Singaraju, (1991); Kharat et al., (2009); Raghava Kumari, (2013); Suryawanshi, (2017) reported that the most alteration of protein in a digestive gland under stress condition. Swami, et al. (1983); Carroll, et al., (2009) and Suryawanshi (2017) recorded a significant decline in protein content in freshwater mussels *Lamellidens marginalis* exposed to the toxicant. There are numerous researchers have made the similar observations in molluscs (Mane et al, 1986; Muley and Mane, et al., 1989; Carroll et al., 2009; Suryawanshi, 2017; Khan et al, 2018). However, the present study indicates that the concentration heavy metal was variable and might be depending on the type of metal, the species of the freshwater mussels *Lamellidens marginalis* and heavy metal in the natural ecosystem. Generally, the variation of metals levels in species of bivalves might be due to body size, reproductive period growth, physiological fitness, and changes in metabolic rate. Therefore the above argument and all the existing literature showed that the Mercuric is very toxic to the freshwater mussels *Lamellidens marginalis*. Hence the release of Cadmium and Mercury compounds in an aquatic environment especially in the freshwater ecosystem need to be controlled.

4. CONCLUSION:

The present investigation revealed that the toxicity of Cadmium and Mercuric on *Lamellidens marginalis* resulted in death within 96 hrs of exposure. The investigations are confirmed that the species has found higher sensitivity to Hg compared to Cd. There is higher protein depletion found in the tissue of digestive glands followed by gills and foots compared to the controlled group. Depletion of protein it would be due to more utilization of these constituents under stress condition of Cd and Hg contamination. This quality monitoring program says that species confirmed the potential to monitor aquatic pollution and can be widely used. The present study is an experimental tool and animal is a bio-indicators for evaluation of aquatic pollution.

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