BIOCHEMICAL ANALYSIS OF WATER QUALITY AND ITS IMPACT ON ZEBRAFISH

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ABSTRACT

The rise in urbanisation and human interference, lake habitats are deteriorating day by day. It was decided to do research on the impacts of Lake, India's water quality. The main aim of the study is Biochemical analysis of water quality and its impact on Zebrafish. Dissolved oxygen (DO), biological oxygen demand (BOD), and chemical oxygen demand (COD) all measured to be 0.0.To sum up, the lake's pollution may have an unfavourable effect on the living things in it.

Keywords: *Urbanisation, Water, Quality, Zebrafish, Dissolved Oxygen, Chemica.l*

1. INTRODUCTION

There has been a rise in the number of contaminants found in aquatic environments; these pollutants are a concern to wildlife and humans alike (1). Rivers and major water sources are polluted by industrial effluent, home waste, sewage, drainage, and pesticides (2), and the bioaccumulation of toxins in aquatic species as a consequence of water pollution poses a rising concern to both wildlife and people (3). As they degrade quickly and are not permanent, organophosphate (OP) chemicals are among the most frequently used pesticides in agriculture and public health, accounting for half of all insecticides used worldwide (4). It has been shown that acetylcholinesterase (AChE) inhibition is a mechanism via which OP poisoning occurs, leading to an increase in acetylcholine levels at peripheral and central nervous system cholinergic receptors (5). It is highly suggested that ecotoxicologists evaluate chemical exposure and consequences using cellular and biochemical criteria (6). Despite the fact that acetylcholinesterase (AChE) has been employed as a bioindicator of exposure to OP pesticides in non-target species (6), oxidative stress has emerged as a crucial molecular mechanism in OP-induced damage. In terms of antioxidant biomarkers, superoxide dismutase (SOD) and catalase(CAT) are rather prevalent (7). A strong antioxidant defence system is essential for cellular homeostasis. In a physiologically normal state, reactive oxygen produced by the metabolism of foreign substances is effectively eliminated by the antioxidant defence system. In populations exposed to pollutants like pesticides, metals, and other xenobiotics, several of these enzymes may be used as effective molecular bioindicators for pollutant-mediated oxidative stress (8). Since toxins build up in food webs, causing harm and eventually mortality in aquatic systems, fish are often employed as a surrogate indicator of ecosystem health (9). In the field of basic physiology, zebrafish are among the most often used vertebrate models. Zebrafish are a good model species for studying toxicity because, despite their tiny size, whole-organ, tissue, or whole-animal analyses are achievable (6).

1.2 The Eco-Toxic Effects of a Common Water Disinfectant on the Swimming Behavior and Recovery Responses of Zebrafish (Danio Rerio)

The need to clean and reuse water resources is a direct result of water scarcity and water pollution. Several waterborne infections and disorders have spread because water treatment systems have failed to effectively inactivate germs. Eliminating harmful microorganisms from water treatment systems and other environments is best accomplished with the use of disinfectants (aquaculture, agriculture, domestic purposes, and health care centres). There were a variety of commercially available disinfectants (a total of 131) that might be used. Due to its powerful antibacterial activity and low cost, chlorine is widely used as a disinfectant. Sodium hypochlorite (NaOCl) is the most widely used chlorine-based disinfectant; it has been effective against microorganisms (including those that are resistant to other disinfectants) since the seventeenth century. This is why NaOCl (up to 9%) is the active component in most common home bleaches. Germicidal products containing NaOCl are widely acknowledged as crucial (US-EPA 1967). They have several uses (in the home, in industry, in aquaculture, in agriculture, in veterinary medicine, in science, and in medicine) (dwell time: 30 s, pathogens: bacteria and viruses). The Environmental Protection Agency (EPA) has approved NaOCl as the active component to inactivate SARS-CoV-2 (EPA listed: 72 products, formulation type: dilutable, wipe, ready to use, Viking, electrostatic spray; surface type: hard nonporous, food contact after rinse necessary). The World Health Organization (WHO) also suggested using NaOCl as a biocide for SARS-CoV, at concentrations of 0.1% for general ambient disinfections and 0.5% for blood spill disinfections. Seventeen species of free-living animals perished in Chongqing, China due to the excessive usage of disinfectants (such as NaOCl). NaOCl decomposes more slowly in the presence of air and more quickly when exposed to bright light, hence its rate of breakdown is largely determined by its environment. Due to its adverse effects on organismal development, the US Environmental Protection Agency (EPA) has classified NaOCl as a Class-I hazardous chemical. Over 80% of effluent (including wastewater from aquaculture) is re-released to environmental compartments without being properly treated, and this is a major cause for worry. After the pandemic, there was an increase in the worldwide use of disinfectants (to 0.78 billion by 2020). Pathogens in polluted areas often need to be neutralised with NaOCl at a high concentration (1000 ppm). As a result, the efficacy of disinfectants and their possible effects on non-target species must be evaluated to guarantee their safety and viability.

The scientific community has developed biomarkers using biological models in response to the growing biological consequences of aquatic pollutants. Ecological risk assessment may benefit from the data gathered from biological monitoring of water pollutants. In the field of aquatic biological monitoring, zebrafish are the go-to model for a wide range of investigations. While useful, conventional biological endpoints have their limits: high-concentration toxicity testing; minimal emphasis on early-warning indicators. The major way in which organisms signal the onset of changes in physiological and ecological processes is via their behavioural activities. So, it is possible to use an organism's behaviour as a reliable biomarker of water quality in an aquatic environment and the toxicity of pollutants in the water itself. Therefore, long-term toxicity monitoring does not need the use of animal sacrifices when using behaviour biomarkers. Fish swim as part of their daily routine, and this habit regulates their ability to do things like find food, avoid predators, have babies, etc. Under duress, a fish's swimming behaviour may change. Hence, the swimming response of fish may be observed at several phases (safe, acclimation, adjustment, or harmful). Possible outcomes of these changes in behaviour include increased or decreased activity levels. In the open environment, fish may become agitated (hyperactive) when under stress. Let's pretend the environment in which fish live is impenetrable. Minimizing gill movement (increasing distance between gill epithelium and the external environment) and water flow allowed the fish to avoid environmental stimuli. Taking this step may cause you to become inactive. Instances like this demonstrate how fish exhibit avoidance behaviour. Interesting avoidance behaviour may show fish's detecting capabilities to stresses. These metrics may help provide risk assessment techniques a more ecological impact. Recovery responses are acknowledged as a useful measure in the field of behavioural toxicology because they show that fish whose behaviour was abnormal as a result of exposure to a toxicant might normalise after they are moved to a new, toxicant-free environment.

2. LITERATURE REVIEW

Bambino, Kathryn & Chu, Jaime (2016) Environmental health and the impacts of toxicant exposure are emerging as major public health problems as manufacturing methods and the production of novel synthetic chemicals proliferate to keep up with the rising worldwide demand. Environmentally occurring substances, such as metals, may also have serious consequences for the wellbeing of humans and other animals. Lead, arsenic, and endocrine disruptors like bisphenol A have all received a lot of attention in recent years for their links to sickness and harm in people and animals. Whereas the potential danger of environmental toxins is generally accepted, the extent to which certain toxins actually contribute to illness is less well understood. When it comes to toxicity studies, zebrafish are at the forefront of the field. This system has been extensively utilised to detect toxins in water samples and to study the mechanisms of action of environmental toxins and associated disorders. The advantages of zebrafish for researching teratogens are similar to those it offers for researching vertebrate development. In this article, we discuss the use of zebrafish in the detection of some toxins and in the identification of the effects of environmental exposures on human health and illness. Here, we zero down on the ways in which zebrafish have been particularly useful for studying ecotoxicology and environmental health, such as in studies of endocrine-disrupting chemicals, byproducts of industrial processes, and arsenic.

Hundt, Matthias & Schreiber (2016) Fish otoliths are often marked with antibiotics like oxytetracycline and tetracycline to determine the success of stocking efforts. Current methods for fish labelling include a variety of issues that might affect mark quality and pose risks to fish during marking. In this research, we look at whether or whether the hardness of the water has a role in the number of freshwater fish that die after being marked with OTC. The spectrophotometric measurement of OTC complexation with $Mg(2+)$ and $Ca(2+)$ cations was performed to investigate this subject. In an added experiment, zebrafish (Danio rerio) were submerged in OTC solutions (1200 mg/L; 48 h immersion) with varied degrees of water hardness (5.5, 15.5, 25.5, 32.5°dH). Water hardness was favourably linked with the quantity of over-the-counter magnesium calcium complexes. It was also shown that zebrafish mortality during marking varied with water hardness. Death rates were higher at the two extremes of the tested labelling with OTC range: 5.5°dH and 32.5°dH.

Liao, Yue & Xu, Jianyu & Wang, Wenjing (2011) The harmful effects of $Cu2+$ at varying concentrations on zebrafish were investigated using an acute toxicity assay that combined biological monitoring techniques with computer vision technologies (Danio rerio). The research established an early warning system based on the quantification of the behavioural reaction of a school of fish in a tank. The system collects and stores quantitative data in real time, and then uses a Multiclass Support Vector Machine (SVM) to perform an all-encompassing evaluation based on the observed behavioural differences between groups of fish in environments with varying levels of toxicity. High levels of accuracy in the predictions suggest that this method is useful for evaluating water quality.

Amado, Lilian & Rosa, Carlos & Castro(2011) The purpose of this research was to assess the state of water quality in the anthropogenically-impacted areas around Patos lagoon in southern Brazil. Five locations were chosen to collect water samples from; one of them was a human consumption source (COR), while the other four were known to be impacted by human activity (IP). Organisms from the genus Danio rerio (Teleostei, Cyprinidae) were subjected to these water samples and a control group for 24 hours. The expression of glutamate-cysteine ligase (catalytic subunit) was greater in COR than in other locations; reactive oxygen species levels were lower in COR and IP than in the control group; and exposure to all water samples had an effect on long-term memory (LTM) compared to the control group. As a result, certain water samples may affect cognitive performance as assessed by LTM and modify the antioxidant system. Water quality in affected areas may be evaluated by using a mix of molecular, biochemical, and behavioural characteristics, as shown by the findings obtained.

Hallare, Arnold & Pagulayan (2005) The influence of current circumstances on aquatic biota is even more important than chemical tests and studies of the water itself if a more thorough evaluation of lake water quality is to be achieved. For example, studies of developmental parameters (96-h embryotoxicity assessment) or of the activation of heat shock proteins may be used as biotests or biomarkers to accomplish this goal (proteotoxicity evaluate). The viability of these assays for use in environmental screening was determined by subjecting fertilised zebrafish eggs to water samples taken from five locations in Laguna Lake, Philippines, where the amount of environmental stress varied. A severely contaminated freshwater source was employed as the positive control, while reconstituted water served as the negative control in the lab. Between 48 and 96 hours of exposure, developmental parameters were observed and characterised. To further evaluate and compare the toxicity potentials of Laguna Lake waters with those coming from a contaminated freshwater, dilution tests of the positive control were also performed. Stress protein (hsp 70) levels were measured in embryos from each group after 96 hours of exposure. Embryos exposed to the undiluted positive control (PC) died at a 100% rate after 12 hours.

3. METHODOLOGY

3.1 Sampling method

a. Physico-chemical and microbiological parameters

Throughout the months of December 2015 through February 2016 from Gardanibag Pond Patna Bihar, three separate composite water samples were collected in clean glass BOD bottles and plastic containers. On-site measurements of pH, temperature, and dissolved oxygen concentration were taken, and the water samples were then sent to a laboratory for further physicochemical and microbiological examination. Standard procedures from APHA (1998), NEERI lab manual Trivedi & Goel were followed to determine physical features like pH, temperature, and total solids, and chemical parameters like DO, BOD, COD, Calcium, Magnesium, Nitrates, Chlorine, Sulfates, Hardness, Total Alkalinity (1986). Standard procedures from the Aneja Laboratory Manual were followed to conduct microbial analysis, including most probable number (MPN) and total microbial counts (2003).

b. Zebra fish analysis

Zebra fish (Danio rerio) were bred from adults bought from fish stores; they were raised in tiny glass aquariums and released to reproduce in the mornings. The collected eggs and embryos were subjected to various dilutions of lake water as well as the control reverse osmosis water. With a high-powered objective microscope, the embryos' unique characteristics were studied and documented, including their size, colour, heart rate, and overall morphology.

4. RESULTS

4.1 Physico-chemical analysis of water

In the present investigation, the pH of the lake was measured, and it ranged from 8.7 to 8.8, with an average of 8.7. The lake's alkalinity may be inferred from this result. The lake's pH has changed as a result of pollution. It has been stated that the pH of a natural lake varies from 4.5 to 6.5. One of the most important abiotic factors in a lake's ability to support life is its temperature. The degree of biological and microbiological activity in water is affected by temperature fluctuations. It modifies dissolved oxygen, acidity, and alkalinity, too. The concentration of oxygen in the water decreases as the water warms. The mean temperature for the research period was 24.1 degrees Celsius, with a range of 21.4% to 29.4%. The hottest month was February, while the coldest was December. The amount of dissolved oxygen in water indicates how much pollution there is from organic matter, while the ability of a body of water to purify itself and the amount of organic matter that can be removed are indicators of its overall quality. Marine life is dependent on dissolved oxygen levels, which fluctuate with water temperature and the number of algae present. When dissolved oxygen levels drop below 2 milligrammes per litre, fish death may occur and the health of a whole ecosystem can be jeopardised. It was determined in this investigation that there was no dissolved oxygen present. The BOD of a body of water is the quantity of oxygen required by microorganisms to break down the organic matter present in the water. There is a correlation between BOD and the amount of organic matter in a given volume of water. Biological oxygen demand affects the amount of DO in water bodies like rivers and lakes. When the oxygen levels in a body of water drop too low, aquatic life dies. This may be caused by excessive levels of COD and BOD. The average BOD reading was 0.0, which is well below World Health Organization guidelines. The chemical oxygen demand (COD) measures how much oxygen is needed to convert the organic matter present in water to an inorganic substance. The research done showed that there was no COD, which is unacceptable according to WHO guidelines. With a mean value of 231.06, calcium levels in the lake were measured to be 232.6 in December, 231.3 in January, and 229.3 in February, with the WHO-recommended limit set at 137.5. The average Magnesium concentration was determined to be 140.66 mg/L, which is within the range recognised as safe by the World Health Organization. Nitrates, chlorine, and sulphates averaged out to safe levels (18.4, 59.53, and 14.36, respectively, according to WHO guidelines). The average hardness of the water was 587.1, which is beyond the threshold set by the World Health Organization, while the total alkalinity was 197.33, which is below it.

4.2 Bacteriological examination of water

The MPN technique was used for the coliform count. Results from the coliform test using the multiple tube fermentation process are often reported using the MPN index. In January, February, and March, we saw elevated MPN levels because bacteria thrived in the warmer temperatures. Coliform populations are indirectly affected by things like over-feeding water bodies, human activity, animal waste, sewage and household waste outflow, and physicochemical characteristics. High levels of MPN suggest that faecal contamination is present. Green metallic sheen found on Eosin Methylene Blue (EMB) agar proved the presence of E. coli. Strains of the colonies were streaked over nutrient agar and stained with gramme stain so that any gram-negative bacteria could be seen under the microscope. The lake water is unfit for human consumption or agricultural use because of the high coliform concentration.

4.3 Zebra fish (Danio rerio) analysis

The zebra fish has served as a valuable vertebrate model for research into the disease's genetics and biological causes. In developmental genetics and neuroscience, zebra fish have shown to be useful models. Ecotoxicological research also makes use of zebra fish. Zebra fish were used to examine the consequences of living in contaminated lake water. In addition to raising zebra fish embryos in contaminated lake water, a control group was also kept. Many concentrations of lake water were produced. When embryos were exposed to the lake water in its purest form, they quickly perished. In table 2 you can see a breakdown of the recorded adjustments.

Table 1: Suggesting likely ranges for December 2014, January 2015, and February 2016.

SamplingSite	Month	NO.oftubesShowingPositive Results			MPN index Per 100ml
		3of10ml	3of1ml	3of0.1ml	
Grab1	December	3	3	3	2400
	January	3	3	3	2400
	February	3	3	3	2400
Grab2	December	3	3	3	2400
	January	3	3	3	2400
	February	3	3	3	2400
Grab3	December	3	3	3	2400
	January	3	3	3	2400
	February	3	3	3	2400

Table 2: Characteristics of Control and Lake Water Zebra Fish Embryos.

CHARACTERISTICS	CONTROL	SAMPLE (10^{-5})
Color	Paleyellow color and highly transparent.	Black colored with minimal transparency.
Shape	Circularouterlayerandinner layer.	Slightlyovoidshapedouterand innerlayer.
Heartbeat	120/min	80/min
Otherabnormalities	Noabnormality	Abnormal growth of spinal cord.

Figure 1 zebra fish embryos

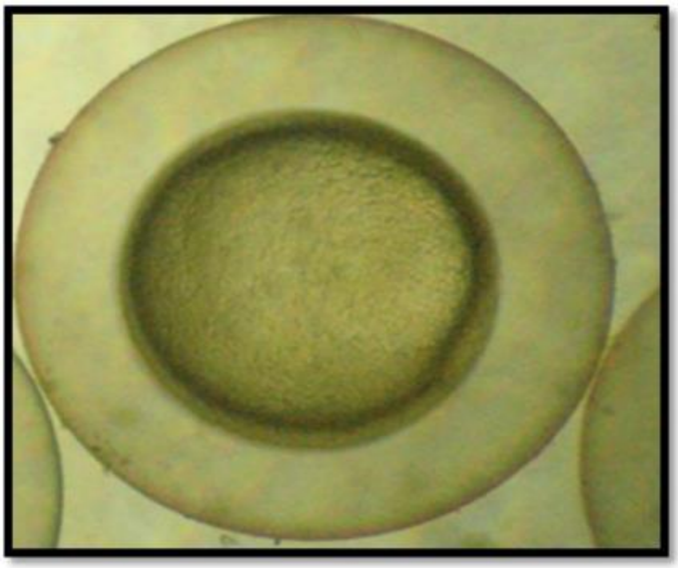
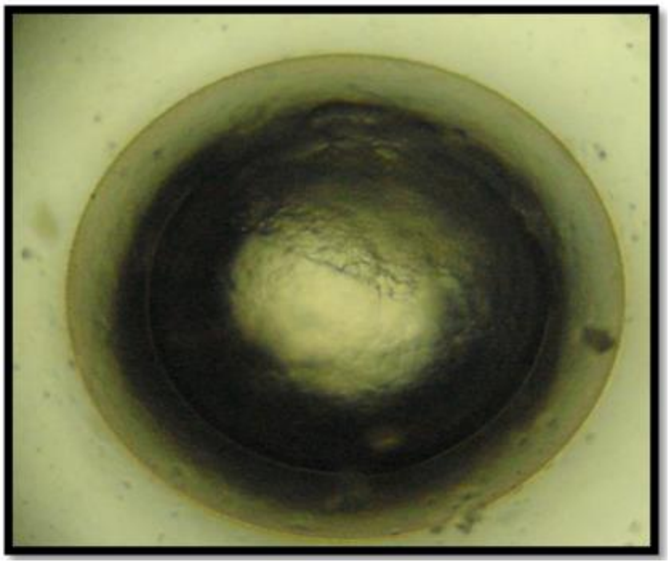


Figure 26 hours later, a fertilised egg may be seen at 10x magnification in a sample of the control water



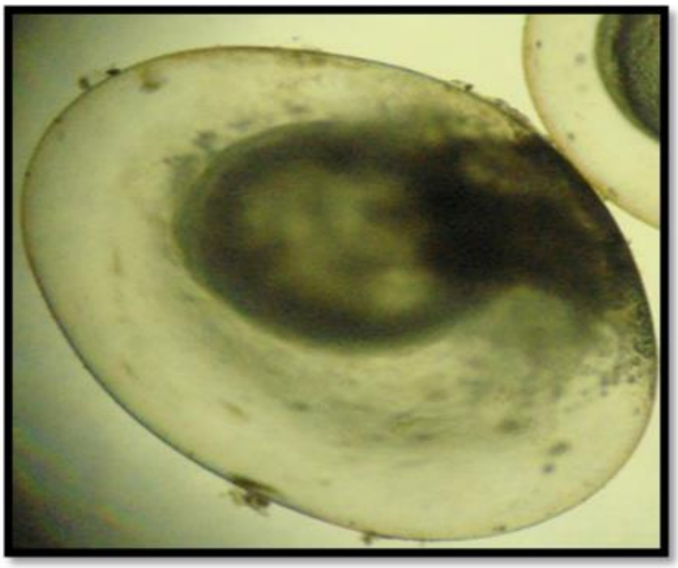


Figure 36 hours after fertilisation, a sample of lake water was examined at 10x magnification to reveal a fertilised egg.

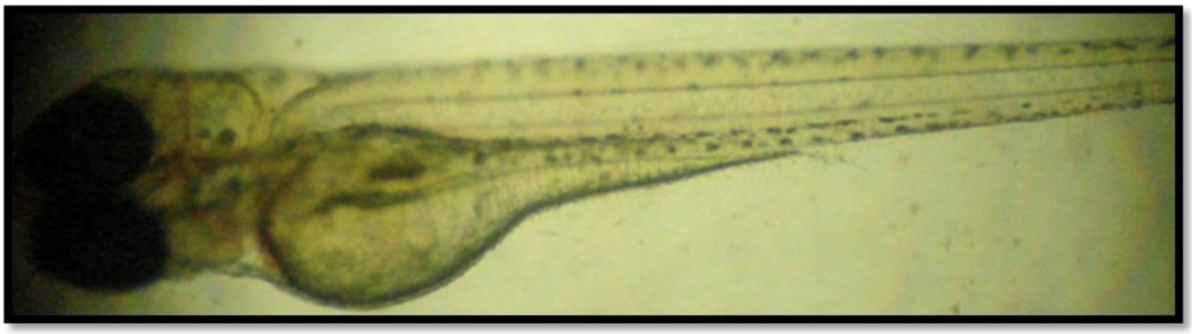


Figure 4Image of a fully formed embryo, taken at 10x magnification from a sample of tap water serving as a control.

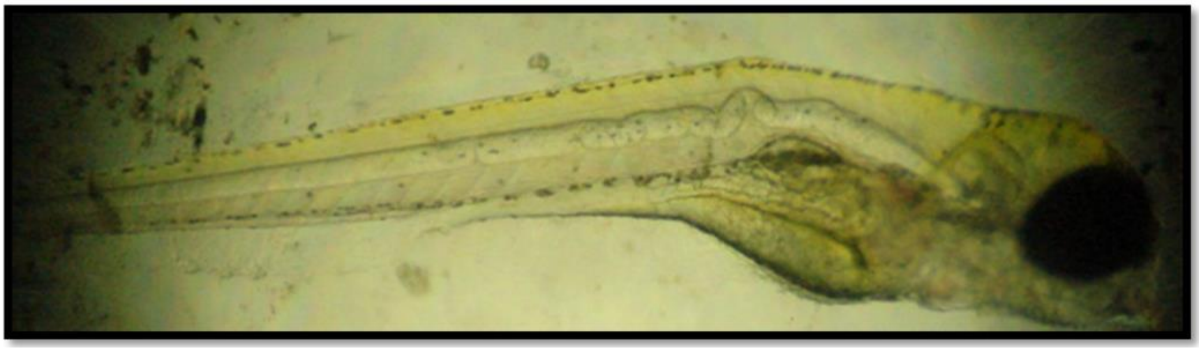


Figure 5magnified view of a fully formed embryo in a 106 dilution of lake water.

5. CONCLUSION

Analysis of the lake water's physicochemical properties showed a shockingly high level of contamination. Although the levels of certain factors (such as pH, calcium, hardness, and total alkalinity) were found to be unsafely high, those of others (such as magnesium, sodium, nitrates, chlorine, and sulphates) were well within the WHO's guidelines. As there was no dissolved oxygen detected in the lake water, both the biological and chemical oxygen demands were likewise determined to be null. This demonstrates that the Lake's water quality is not suitable for marine life. The most probable number (MPN) index per 100 ml of the sample was determined to be maximal, and the presence of coliform bacteria was verified using EMB and nutritional agar medium, indicating that the water could not be utilised for home and agricultural reasons. The results of eco-toxicological tests conducted on the invertebrate system of zebra fish are shown in table 2. Undiluted water sample killed zebra fish embryos, but diluting it by a factor of 106 caused dramatic changes in the embryo's heart rate and overall shape, making it viable. The fish's spinal cord seemed to have undergone a dramatic transformation. Using lake water may have the same or even more dramatic effects on higher species. This proves beyond a reasonable doubt that it would be impossible for any kind of life to persist in lake water.

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