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Impact of Agriculture Fertilizer Containing Nitrogen on Oxygen Consumption of Freshwater Fish; *Tilapia mossambica*

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Abstract: ***In present days excessive fertilizers are using in the agriculture fields to get good crops. Some times these fertilizers enter in to fresh water bodies through run off with rain water. Fishes living in these fresh water bodies which are surrounded by these fields have to face problems evoked by nitrogen containing fertilizers and other fertilizers. Hence present investigation is to study the effect of nitrogen contained fertilizers on oxygen consumption of the fresh water fish Tilapiamossambica. The effect of nitrogen - containing chemical fertilizers like urea on oxygen consumption in Tilapia mossambica was investigated. Fishes were collected from Belur Pond in the Belur village of Dharwad taluk of Karnataka, India. Fishes were exposed to different urea concentrations for 96 hours to investigate oxygen consumption by fish in the water. Dissolved oxygen was carried out by Wrinkle's titrimetric method before and after the administration of fish into different concentrations of urea. LC50 value was detected. The urea concentrations prepared were 0g/lit (control), 3g/lit, 6g/lit, 9g/lit, 12g/lit, and 15 g/lit. Fishes were exposed to different urea concentrations in separate aquariums for 96 hour. After 96 hours, dissolved oxygen consumed by the fishes in control and aquariums containing different urea concentrations were estimated. It was investigated that the amount of oxygen consumption by fishes decreased if urea concentration was increased. Furthermore, it was investigated that the mortality of fingerlings increases with increasing urea concentrations. Thus it was shown that urea affects the oxygen consumption of fish in freshwater ecosystems. The present investigation showed that an increase in urea concentration might result in decreased oxygen consumption***

Keywords: Urea, Freshwater, Belur, Dissolved oxgen,, Ecosystems

1. Introduction

Water is one of the supreme elements accountable for life on earth; regrettably, countless of its water bodies are becoming increasingly dark, blocked with the growth of algae, and smelly due to the contamination of several chemicals such as detergents, fertilizers, pesticides, heavy metals, sewage and many wastes were discharged from several industrial units along with this drainage from the cities changing the lifestyle. Consequently, these hazardous chemicals appear acute toxicities for many aquatic organisms. As a result, many fish species have failed to survive the pressure caused by agumanted concentrations of various chemicals discharged into the water bodies (Sheela, Letha, and Joseph 2011) **.** More accumulation of fertilizers and pesticides have created these water bodies unsuitable for human use. Several studies and results have published the effects of pesticides and fertilizers on the fauna and flora of aquatic ecosystems (Aktar, Sengupta, and Chowdhury 2009) **.** Maintaining the healthy nature of freshwater bodies declined faster due to anthropogenic activities like discharge of chemical fertilizers and sewage into the water and habitat degradation. Hence it is very much necessary to maintain fresh water bodies and healthy fish population. The release of fertilizers may affect the oxygen consumption capacity of fish (Mathew, Sunitha, and Thomas 2013) **.** To survive for a more extended period all fish require oxygen. If dissolved oxygen levels come down, a few sensitive animals may die or move away and become weakened; studies on dissolved oxygen content of water and its consumption by fish attracted the scientific community's attention, a few fishery workers, and researchers in this context. We have chosen freshwater fish *Tilapia mossambica. (Oreochromis mossambicus)* in the present investigation. **.** This fish is an excellent species for aquaculture because it is immediately available and can adapt to a new situations. Because of its euryhaline nature and this species has assumed an excellent model for studying the mechanism of osmoregulation in teleost fishes (Evans 1984) **.** Aften fish act as a biological indicator to determine the quality of water. If anything changes, it happens in fish physiology, which indicates deterioration of water quality conditions. Hazardous chemicals, particularly pesticides, could spoil the aquatic environment, leading to several changes in organisms physiology (Schlenk 2005) **.** So, the evaluation of oxygen consumption is the critical parameter to evaluate pollution stress. Furthermore, determination of oxygen consumption not only observed metabolic rate but also furnished an index for fish stress conditions. Fish can absorb pesticides via alimentary ducts, skin, and gills (Mahdi 2012, 2013).

Many studies indicate the mechanism through which chemicals can affect fish organs and the percentage of oxygen consumption (Oti EE 1997, Rani Rekha 2008) **.** It is well known that fish breathe dissolved oxygen in water through the gills, which comprise the gill filaments that absorb dissolved oxygen through them. Furthermore, pesticides affect the histological structure and physiological function of these gills, such as necrosis and hyperplasia of gill filament cells and fusion of secondary lamellae as a result of this, which leads to affect the process of oxygen consumption (Velmurugan, Mathews, and Cengiz 2009, Bhuvaneshwari, Padmanaban, and Babu Rajendran 2015) **.** Oxygen consumption plays a significant role in fish

physiology. It is an index of fish metabolic rate. Several factors, like fish size, water temperature, and seasonal variations, influence the oxygen consumption in fish (A.2015) **.** The physiological and behavioral alterations occur in fishes in the absence of a sufficient amount of oxygen (Ch. Prasanna1 2020) **.** Today, we adopted modern agricultural practices using general mineral fertilizers. However, these fertilizers are biodegradable but have a slow rate of degradation because this reason increases their pollution potential (Chouhan 1987.) By - products of nitrogenous fertilizers are dangerous and toxic to several species of fish (Palanichamy 1985) **.** Freshwater ecosystems are facing several anthropogenic pressure. The increased levels of grazing and fishing activities have also resulted in the degradation of wetlands (S. N. prasadi andvijayani 2002) **.** The fish fauna is the most crucial source of the freshwater ecosystem. Fishes contain many proteins and vitamins (Tanveer et al.2015) , fish fauna is affected by the use of wetlands on a large scale, improper use of fresh water, and release of sewage and agricultural fertilizers into freshwater ecosystems. Different fertilizers are generally used to get good crops, but these fertilizers cause lethal effects on aquatic organisms like fish and other zooplankton. These fertilizers dissolve and wash into freshwater bodies through rain, affecting freshwater flora and fauna (Ajima et al.2015) **.** Urea [NH_2 - CO - NH_2] is a widely used fertilizer; hence, this fertilizer is washed with rainwater and regularly enters freshwater ecosystems. Fertilizers like urea in water bodies may decline fish production and cause fish death (Jhingran 2005) **.** Urea increases the growth of phytoplankton and weeds in water bodies, leading to a decline in the amount of oxygen in freshwater resources; this may lead to the mortality of fishes and other aquatic organisms (Omoregie, Ajima, and Keke 2003) **.** Maintaining the healthy nature of freshwater bodies declined faster due to anthropogenic activities like the discharge of chemical fertilizers into the water and habitat degradation. Hence it is very much necessary to maintain a healthy fish population. The release of fertilizers may affect the oxygen consumption capacity of fish (Mathew, Sunitha, and Thomas 2013) . Furthermore, the discharge of agricultural fertilizers like Nitrogen**,** phosphorus, and potassium, or NPK, may also affect the physiology of fish (Asuquo IE, Essien - Ibok MA, and NO3 2016) **.** Using agricultural fertilizers like urea affects fish's oxygen consumption capacity (Palanivelu et al.2005) **.** These nitrogenous compounds can result in toxic effects on aquatic vertebrates. Discharge of urea fertilizers affects the growth rate of fish, and the growth rate is generally reduced (Palanivelu et al.2005) . Indiscriminate use of fertilizers like urea may change water's PH and change water's physicochemical parameters, thus affecting fish production (Hunt and Boyd 1981) . Urea is one of the nitrogenous fertilizers that enters surface and ground water through leaching and surface runoff from agricultural lands. Its entry into groundwater depends on soil texture (L 2012) **.** Urea is known to hydrolyze efficiently in to water to give ammonium carbonate, which is volatile and thus releases ammonia (Onusiriuka 1992) . Agricultural fertilizers, pesticides, insecticides, and industrial effluents pollute water bodies and alter the ecological balance. Fertilizes contain organic or inorganic, natural or synthetic materials which are very much necessary for plant growth NPK and Urea (Amadi. A 1991) **.** fertilizers are carried through surface runoff from cultivated agricultural forms, entering into freshwater fertilizers and affecting fish diversity. The fertilizers enter freshwater or aquatic ecosystems through super phosphate production effluent, runoff, and discharge from industrial effluents (Long 1978) **.** Thus the present study aimed to evaluate urea fertilizers' effect on the oxygen consumption of *Tilapia mossambica*.

2. Materials and Methods

Fingerlings of *Tilapia mossambica* with a mean body weight of about 10 gm +/ - 0.5 gm were collected from the Belur pond of Dharwad taluk of Dharwad district, Karnataka state. The young fishes collected were stocked in aquariums measuring 50*30*30 cm3 with chlorine - free municipal tap water, which is well aerated. Furthermore, these fishes were acclimatized for 14 days (Ofojekwu, Nwani, and Ihere 2008a) under laboratory conditions. Acclimation of these fishes was necessary to enable them to recover from collection and transportation stress and fishes were made stable for experiments. These fishes were fed daily twice (08: 00h and 16: 00h) with 40% crude protein pelleted fish food at five percent of body weight. Mortality of these fingerlings was observed during the acclimation period. Mortality was observed at less than 2% during the acclimation period. Mortality of fingerlings was recorded after 24, 48, 72, and 96hr (kumari 2021) . After acclimation, the mortality of fingerlings was studied by preparing urea concentrations of 15g/l to 45g/l to record mortality between 24 - 96 hrs. At urea. Finally, LC 50 value obtained was 18.26 gms/lit. Different urea concentrations were prepared using the serial dilution method of Warner (1962). Sub lethal urea concentrations were prepared and used for experiments 0gm/lit (control), 3gm/lit, 6gm/lit, 9gm/lit, 12gm/lit, and 15gm/lit in dechlorinated tap water. Different urea concentrations were prepared in 10 liters of tap water used for preliminary runs until proper concentration, resulting in 100% mortality within 72 hrs. This solution was considered a stock solution. (Rani, Elumalai, and Balasubramanian 1997) . Then a group of 5 fish was transferred into six aquariums (Palanivelu et al.2005) **,** each measuring the size of 50*30*30 cms3. Aquariums were marked as A, B, C, D, E, and F. Each aquarium contained 10 liters of dechlorinated tap water with different concentrations of urea fertilizer. Aquarium " A" was considered as control with zero urea concentration, aquarium "B" with urea concentration of 3gms/lit, aquarium "C" with6gms/lit, aquarium "D" with 9gms/lit, aquarium "E" with 12gms/lit, aquarium "F" with 15gms/lit. L. C.50 values of urea of these fishes were detected by exposing a group of 10 fingerlings for 96 hrs to different urea concentrations. For 96 hrs of exposure, L. C.50 value obtained was 18.26gms/lit. It was taken to a sub - lethal level. The fishes were exposed to urea concentrations below sub - lethal level. The L. C.50 values may be varied from one investigation to another due to the size of fishes, age, temperature, hardness, pH, fish species, etc (Palanivelu et al.2005).

The toxicity of fertilizer depends on the concentrations of free molecular NH_2 (Rani, Elumalai, and Balasubramanian 1997) . The maximum urea concentration of 18.26gms/lit showed 100% survival of fish. This concentration was considered a sub - lethal level. Thus below this sub - lethal

concentration was taken as the maximum concentration for experiments. The aquarium's water was changed regularly to remove fecal matter and dead fingerlings. These fishes were exposed to these six different concentrations for 96 hrs, and the experiment was conducted in a replicate. Experimental fishes were not fed 24 hrs prior to the experiment. During 96 hrs of the exposure period, the test water of each aquarium was drained out thoroughly every morning. Fingerlings were removed carefully with the help of a scoop net. After placing test water with urea concentration in aquariums, these fishes were placed back into their respective aquariums. Before the release of fish into each aquarium, the dissolved oxygen of each aquarium was estimated with Winklers volumetric method (Welch 2002) **.** Then fishes were released into the aquarium for 96 hours. After 96 hours, water samples from each aquarium were collected, again estimating their dissolved oxygen content. The difference between the amount of dissolved oxygen in the water sample before and after 96 hours was noted. It gives the average oxygen consumption of the fish in each aquarium. Dissolved oxygen consumption was estimated in control and urea - exposed fishes by placing them in a respiratory chamber containing test water. (Palanivelu et al.2005) . The amount of dissolved oxygen consumption was calculated per gram of body weight per hr. (Neelima et al.2016) (Table2). D. O. of the aquarium "A" with control was also estimated after 96 hours. The whole experiment was repeated twice. Physicochemical parameters of water were regularly monitored every 24 hrs by applying methods of APHA (1998) ; American Public Health Association. In; standerd methods for the examination of water and waste water. APHA/AWWA/WPCF).

Study Area:

Belur pond (15 30’55.6”) N latitude and (74 55’ 13.7”) E longitude situated in Belur village of Dharwad taluka, in Dharwad district of Karnataka state, India. It is located 10 km towards North - West of Dharwad and 435 km from the state capital, Bangalore. This pond has clean water for irrigation, fishing, and domestic purposes.

3. Results and Discussion

Results

All aquariums' dissolved oxygen was estimated before releasing fishes into aquariums. The D. O. of water of aquarium "A" that is, control was 7.61mg/lit. This reading was taken as control. Furthermore, this D. O. content was considered sufficient D. O. for the survival of fish. After 96 hours, D O concentration was recorded as 7.14mg/lit in aquarium "B" with 3 mg/lit urea concentrations. D O concentration was 6.89mg/lit in aquarium "C" with 6mg/lit urea concentrations. Furthermore, D O concentration was 6.02 mg/lit in aquarium "D" with 9mg/lit urea. Moreover, D O concentration was 5.80mg/lit in aquarium "E" with 12mg/lit urea concentrations. Moreover, D O concentrations were 5.02 mg/lit in aquarium "F" with urea concentrations of 15mg/lit (Table2 and Fig 2). *Tilapia mossambica* exposed to 96hrs LC50. It was recorded at 18.26g/l (probit Y=3.28) +X=7.68). lower confidence limits of 9.17 g/l and upper confidence limits of 26.5g/l. A positive correlation was formed in the graph of logarithmic fertilizer versus probit mortality (Ofojekwu, Nwani, and Ihere 2008b) **.** This indicated that as the urea fertilizer concentration increased, the mortality rate increased. (**Table 2 and Figure 2**) At urea 40 g/l concentration, 100% mortality was observed within a 24 - 96 hrs exposure period. At a concentration of 35g/l, mortality was 90%and at concentration.30g/l mortality was 80%. At 25%, mortality was observed only 30%. At a concentration of 20g/l, mortality was 10%—no mortality was observed at a concentration of 15g/l and below. (**Table: 1 and Figure 1).** This result obtained was the variance of findings of other investigators because this difference may be due to differences in the fish species, the difference in the weight of fish, the difference in physico chemical properties of test water, feeding of fish, the pollution level of water. The mean mortality of fingerlings exposed to different urea concentrations caused significant ($p<0.05$) but observed variable death rates in exposed fingerlings. The mortality rate observed was directly correlated with urea concentration gradients. At higher concentrations, the highest death rates were reported, which were significantly higher than the remaining concentrations. These findings followed the findings of Asuquo et; al (2016) (Asuquo IE, Essien - Ibok MA, and NO3 2016) **,** who worked on the effect of some agricultural fertilizers on fingerlings of *heterobranchus bidorsalis.* Nevertheless, no mortality was observed in the control groups. Oxygen consumption of fishes *Tilapia mossambica* was investigated. It was calculated per gram body weight of fish. Values of results were expressed graphically. It was observed that oxygen consumption was slightly more during the initial exposure time in the initial four hours. Then it was observed that there was a continuous decrease in oxygen consumption till the end of the experiment. This investigation follows the findings of P Neelima et; al (2016) (P. Neelima 2016) **.** who worked on the effect of other toxins on *Cyprinus carpio.* A decrease in oxygen consumption with the time of exposure to the urea toxicant was observed in this investigation, following the findings of Tilak and Swarnakumari (2009), (Tilak and Kumari 2009) , who worked on some other toxins in fishes. As urea concentration increased, it was observed that fishes showed behavioral changes like respiratory distress, rapid operculum movements, erratic swimming, and attempts to jump from the aquarium.

Furthermore, physicochemical parameters were regularly monitored every 24 hrs of exposure time. Physico - chemical parameters showed a decrease in dissolved oxygen concentration. However, it was recorded that total dissolved solids, total alkalinity, total hardness, salinity, and organic nitrogen showed an increase in concentration **(Table: 3).** But it was recorded that no significant difference was observed between values of temperature and PH ($P>0.05$). these findings follow the findings of Ofojekwu. P. C. (2008) (Ofojekwu, Nwani, and Ihere 2008b). Who worked on the acute toxicity of urea fertilizer to Tilapia Zilli fingerlings. Oxygen consumption may vary from one investigation to another because it may depend on the type of fish species, food intake capacity of fishes, pollution level of water, and changes in physical, and chemical properties of water.

4. Discussion

In the present investigation, it was observed that as concentration increases, the mortality rate increases. This is because urea hydrolyses easily in water and releases ammonium carbonate. As this ammonium carbonate is volatile, it releases ammonia (Onusiriuka 1992) **.** As urea fertilizer concentration increases, the consumption of oxygen by fish decreases. However, at the initial time, oxygen consumption showed slightly more; afterward, oxygen consumption continuously decreased until the end of the investigation. The initial increase in oxygen consumption may be due to stress caused by urea toxicant on the fish, which causes activeness to combat stress, hence incurring an increased energy requirement**.** (P. Neelima 2016) **.** During sub - lethal concentrations, at the initial stage, due to an increased amount of toxins, fishes breathe faster than usual hence at the initial stage, it was observed increase in oxygen consumption (Veeraiah 2001) worked on the effect of toxins (cypermethrin) on *Labio rohita.* The findings of the present investigation follow the findings of (Veeraiah 2001) **.** These findings of the present investigation also follow the findigs of (Shereena, Logaswamy, and Sunitha 2009) **.** who worked on *Tilapia mossambica* exposed to *dimethoate.* However, it was later shown that decreased oxygen consumption by fishes due to the entry of urea molecules might lead to decreased energy requirements (Tilak 2002) **.** During toxic conditions, the nature of water in aquariums becomes more toxic due to increasing the amount of urea concentration. Hence it is not easy to consume oxygen properly by fishes (Tilak and Kumari 2009) . As the concentration of urea increases, fishes show respiratory distress, rapid opercular movements, increased mucous secretion restlessness, and erratic swimming. This study follows the findings of (Ofojekwu, Nwani, and Ihere 2008b) . (Avoaji 1997) , (Omoregie, Ajima, and Keke 2003) **.** These fishes at higher urea concentrations also started to engulf oxygen through the mouth. Water with urea concentration comes in contact with fish gill surfaces, and these toxins may damage gill surfaces which may lead to respiratory distress (Magare 2000) . Deposition of mucous on gill surfaces may reduce oxygen diffusion, which may reduce oxygen consumption by fish. (P. Neelima 2016) **.** Physico - chemical parameters showed some changes during the investigation period. It was recorded that dissolved oxygen was shown to decrease concentration, but the remaining parameters showed slightly increased concentration. No significant difference was observed in PH and temperature. During the present investigation, some behavioral changes in fishes in aquariums B to F were observed continuously for 96 hours. It was observed that urea severely impacted the oxygen consumption of fish. With the increased concentration, fish showed increased breathing, and signs of distress were observed. Fishes showed discomfort within 30 minutes and started moving circularly with increased urea concentrations. It was observed that the breathing and movement of fishes increased by increasing urea concentrations in different aquariums. The fish showed fast breathing, and mucous on the body surface emerged. Moreover, in 96 hours, fishes become inactive and rest at the bottom of the aquarium. No behavioral changes were observed in the aquarium with the control, and no changes were observed in oxygen consumption. Mean D O consumption per gm body weight of the fishes used in the experiments was calculated and shown in Table 1

Table 1: Different urea concentration and percentage of survival of fingerlings

Urea concentration (g/l)	Percentage of survival of fingerlings after 24, 48, 72, 96 hours exposure			
	24h	48h	72h	96h
15	100	100	100	100
20	100	100	100	90
25	90	80	80	70
30	60	40	30	20
35	50	30	30	10
40	00	00	00	00
45	00	00	00	00

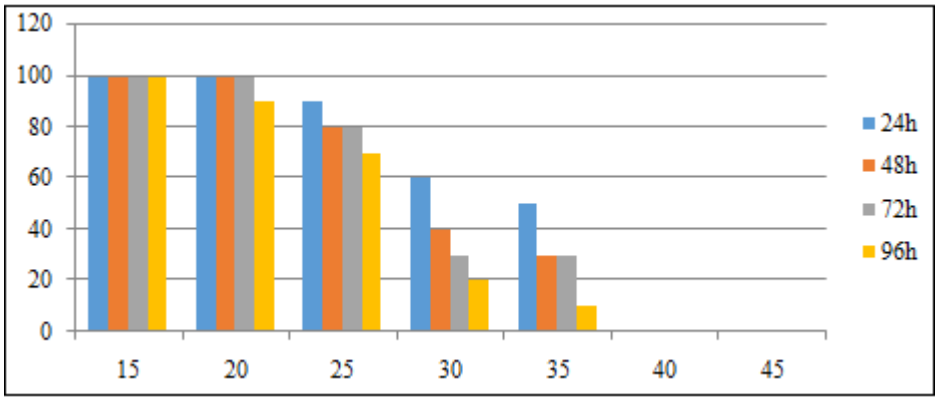

Figure 1: Different urea concentration and percentage of survival of fingerlings and Urea concentration 15, 20, 25, 30, 35, 40, 45 (g/l)

Table 2: Difference in Dissolved oxygen levels under the treatment with different sub lethal Urea concentrations: (D. O values of 96 hr, mean of all 2 independent experiments)

Concentrations Of urea (g/l)	D. O mg/lit Consumed by fish	D. O consumption mg/lit/hr of body weight
0 g/lit	7.61	0.76
3 g/lit	7.14	0.71
6 g/lit	6.89	0.68
9 g/lit	6.02	0.60
12g/lit	5.80	0.58
15 g/lit	5.02	0.50

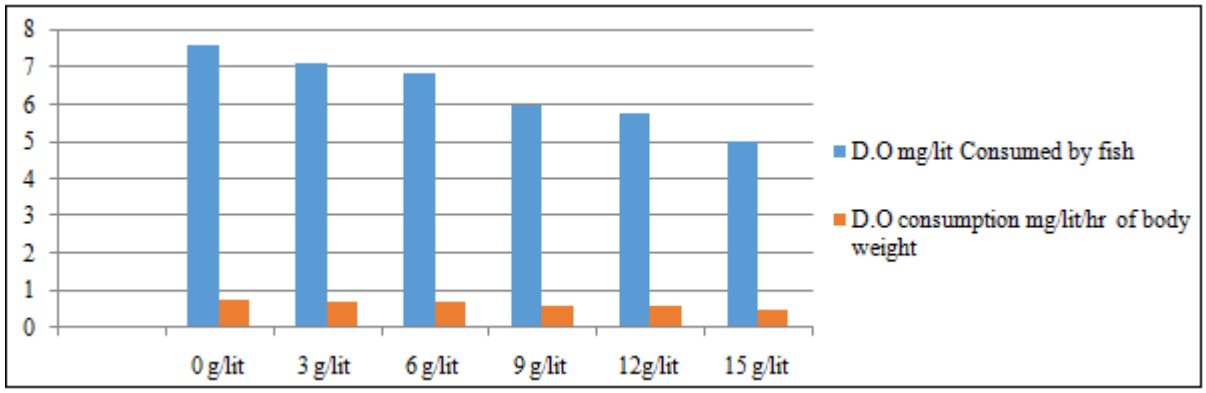

Figure 2: Difference in Dissolved oxyegen levels under the treatment with different sub lethal Urea concentrations: (D. O values of 96 hr, mean of all 2 independent experiments)

Table 3: Mean water quality parameters during 96 hrs exposure of *Tilapia mossambica* to ac cute concentrations of urea

Parameters	0 g/l	5g/l	10g/l	15g/l	20g/l	25g/l
Temperature (^{O}c)	25.9	26.20	26.22	26.80	26.57	26.01
Dissolved oxygen (mg/l)	7.61	7.14	6.89	6.02	5.80	5.02
Total hardness (mg/l)	17.2	17.4	17.42	17.8	17.4	17.5
$_{P}$H	6.92	6.5	6.1	6.91	6.61	6.51
Alkalinity (mg/l)	16.32	16.41	16.40	16.45	16.50	16.60

5. Conclusion

Chemical fertilizers containing urea are generally used for plant growth in fields. Urea is an excellent chemical fertilizer for plant growth, hence urea can be used for proper growth of crops. These fertilizers better not to allowed to enter in to near by fresh water bodies. These fertilizers better to use for proper maintenance of crops. Sometimes these fertilizers may enter nearby freshwater bodies through water runoff, rain, and any other ways of leakage, thus diminishing water quality and affecting the life of aquatic organisms like fishes and often zooplankton. These chemical fertilizers enter into aquatic bodies in soluble or particulate forms and deliver soluble phosphorous, nitrogen, and carbon (Cooke G W. Fertilizing for maximum yield. crosby Lockwood Staples) . Urea [NH_2 - CO - NH_2] generally contains nitrogen; some research investigated that such fertilizers have been found to affect fish health, particularly effect on oxygen consumption in fishes. The present investigation showed that an increase in urea concentration in fresh water bodies might result in decreased oxygen consumption, behavioral changes, fishes showing discomfort, and coming down to the bottom of the aquarium. Their breathing rate was also decreased, thus resulting in decreased consumption of oxygen. Hence it was concluded that freshwater bodies should be protected from entry of chemical fertilizers. Care should be taken not to enter chemical fertilizers into fresh water bodies. Thus, there will be proper growth of fishes and other aquatic organisms in freshwater bodies.

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Conflict of interest
The authors have declared no conflict of interest

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