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Application of Micro-nutrients Affects Planktonic Quality and Quantity

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Abstract

An experiment was conducted for 90 days in outdoor, circular, 1000 L, cement cisterns with soil base for evaluating the effect of nutrient supplementation on planktonic quality and quantity. All the cisterns were manured with cow dung (400 g), Urea (1 g) and single super phosphate (1.5 g) at fortnightly intervals. Water-soluble forms of Magnesium (Mg), Manganese (Mn), Zinc (Zn) (100 mg each) and Silicon (Si, 0.2 ml) were applied separately to cisterns, maintained in triplicate for each treatment, once in 10 days. Planktonic quality and quantity and water quality were analysed at monthly intervals. The population of diatoms, rotifers and plankton belonging to Chlorophyceae, Euglenophyceae and Trebouxiophyceae were the highest in tanks supplemented with Si. The next higher density of plankton belonging to Trebouxiophyceae was recorded in Mg treatment. The highest density of planktonic Cyanophyceae was recorded in Zn treatment. The density of planktonic crustacean was higher in control and Mn treatment. Excepting a lower yield with Zn treatment, the plankton biomass recorded during the experimental period in different treatment tanks did not differ significantly. The study revealed that the supplementation of nutrients to the culture system affects the taxonomic quality of plankton produced.

Keywords: Micro-nutrients, plankton, silicon, zinc, magnesium

Introduction

The success in producing fish fingerlings for stocking in the grow-out production system is essentially hooked into the supply of suitable live food for young fish. Production of live food organisms continues to be an essential requirement in the intensification of aquaculture. Live food organisms contain all the nutrients like proteins, lipids, carbohydrates, vitamins, minerals, amino acids and fatty acids (New, 1999) and hence are commonly referred to as “living capsules of nutrition”. Promoting the growth of live feed such as phytoplankton and zooplankton in aquaculture systems can minimize nutrient deficiency, reduce dependence on formulated feed and control eutrophication.

Both the qualitative and quantitative abundance of plankton in a culture pond is of great importance in managing successful aquaculture operations. Phytoplankton forms the base of the food chain in pond culture, grows utilizing the available nutrients and zooplankton feed on them, which will in turn be consumed by fish. Further, phytoplankton plays a pivotal role in maintaining water quality by effectively maintaining oxygen levels, light regimes, bacterial numbers as well as zooplankton biomass and assimilating ammonia generated by fish excretion (Lorenzen et al., 1997). In addition to carbon dioxide, water and sunlight, phytoplankton needs mineral elements for carbohydrate synthesis. Natural productivity of nurseries is often unsatisfactory due to a deficiency of one or more of the minerals in soil and water. Correction of deficiencies by application of manure or fertilizer containing these nutrients in suitable form and optimal quantity is an essential, cheapest and simplest means for

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efficient pond management to increase the production of live food organisms. These fertilizers are used mainly to increase the availability of the major nutrients, nitrogen and phosphorus, in pond water, which are needed for phytoplankton growth. Inorganic nitrogen and phosphorus are the nutrients considered important in regulating the growth of phytoplankton in freshwater ponds (Ornolfsdottir et al., 2004). However, other micro-nutrients, such as calcium, sulfur, iron, manganese, copper, boron, magnesium and zinc are also required for their growth and nutrition (Boyd & Scarsbrook, 1974). The major nutrients and micronutrients appear to have fundamentally different effects on the growth and species composition of phytoplankton. The micro-nutrients had marked effects on the species composition while major nutrients had minimal effects on the same (Frey & Small, 1980; Sunda, 1989).

There are some studies evaluating the effect of nutrient enrichment on the phytoplankton populations (Glooschenko & Curl, 1971), but reliable methods for controlling the species composition of phytoplankton communities have not been developed (Boyd & Tucker, 1998; Mischke, 2012). The frustules of diatoms, the hard but porous cell walls, are composed almost entirely of silica (silicon dioxide) (Annenkov et al., 2020; Boyd, 2014). Silicon (Si) in the form of calcium silicate and sodium silicate has been shown to stimulate diatom growth in ponds with low silicate concentrations (Daniels & Boyd, 1993). Li et al. (2007) opined that Si dynamics could affect the phytoplankton dominance, food web and biogeochemical cycling in both marine and freshwater ecosystems. Freshwater diatoms usually need more Si than marine diatoms (Nagai et al., 2001). Zinc (Zn) is a structural element of proteins involved in gene regulation, translation and DNA repair and also functions as Lewis acid in biochemical reactions. Zn may also be an important limiting factor for phytoplankton growth (Chappell *et al.* 2016). Adhikari & Ayyappan (2004) reported that incorporation of zinc in soil improved the plankton productivity in freshwater farms. Magnesium requirement for phytoplankton is less than 2 mg L^{-1} (Boyd, 2015). Magnesium along with calcium is considered to be very important for moulting and new shell formation in shrimps. There are only a few studies examining the effects of manganese limitation even though manganese is an important component of the photosynthesis apparatus. There is scarcity of literature on the effect of enrichment/supplementation of freshwater aquaculture systems with mineral nutrients in general and Silicon (Si) in particular on the planktonic quality and quantity. The present experiment evaluated the effect of supplementation of culture water with soluble forms of Mn, Mg, Zn and Si on planktonic quality and quantity.

Materials and Methods

The experiments were conducted for 90 days in outdoor, circular, 1000 L, cement cisterns. The cisterns were provided with 3 inch thick soil base. The nutrient content of soil samples from tanks were analysed before initial manuring following standard methods [pH and electrical conductivity (Jackson, 1973), organic carbon (Walkley & Black 1934), available nitrogen (Subbiah & Asija, 1956), available phosphorus (Bray & Kurtz, 1945), available potassium (Hanwey & Heidel, 1952), exchangeable calcium and magnesium (Lindsay & Norvell, 1978), silicon (Narayanaswamy & Prakash, 2009)]. Analysis of the soil samples from each tank was also carried out after the experiment. Analysis of silicon in the soil before the experiment could be conducted only with one soil sample for technical reasons. However, it may be noted that the source of soil for all the experimental cisterns was the same. All the cisterns were manured with cow dung (400 g), Urea (1 g) and single super phosphate (1.5 g) at fortnightly intervals (CIFA, 2009). For nutrient analysis, the manure was dried at 70 ºC for 24 h and ground to pass through a 20 mesh sieve. One gram sample of ground manure was dry ashed at 450 ºC for 2 h. Ashed samples were extracted with 0.5 N HCl. Nutrient concentrations were determined according to Jackson (1973). There were four treatments in total with each treatment supplemented with any of the following micronutrients; $MgSO_4$ (Mg), $MnSO_4$ (Mn), $ZnSO_4$ (Zn) (all at 100 mg m^{-3}) and silicic acid (H_4SiO_4) (2 % ortho silicic acid [Si] dissolved in polyethylene glycol @ 0.2 ml m^{-3}), maintained in triplicate and supplemented every 10 days. Tanks which did not receive any nutrient served as control. Water quality and planktonic quality (species composition) and quantity were analysed at monthly intervals (APHA, 2005). For plankton analysis, samples were collected by filtering 100 litres of tank water using 15 micron fabric mesh. The filtered water was poured back to the same tank. The filtrate containing plankton was immediately preserved in buffered formalin (5 %) in plastic vials and was later enumerated for species composition to generic level using Sedgwick Rafter Cell (Jhingran et al., 1969) and was finally grouped into different classes for comparison. Plankton biomass was also estimated using the same sample after enumeration.

Statistical analysis of data on water quality was done by the analysis of variance (ANOVA) at p=0.05, followed by Duncan's multiple range tests wherever required (Duncan, 1955).

Results and Discussion

The nutrient content of soil samples of tanks analysed prior to and after the experiment are given in Tables 1 and 2. No major differences in nutrients were observed between the samples, possibly because the soil used in all the experimental tanks was taken from the same location. However, the analysis indicated a reduction in available P and an increase in S and Fe at the end of the study, irrespective of the treatments. An increase in the Mn and Zn content was also recorded in soil samples from the respective treatments.

The water quality parameters recorded during the study period showed no significant difference ($p>0.05$) between the treatments (Tables 3). Qualitative estimation of plankton revealed the following. The population of diatoms (Bacillariophyceae and Coscinodiscophyceae), rotifers and plankton belonging to the Classes Chlorophyceae, Euglenophyceae and Trebouxiophyceae were the highest in tanks supplemented with Si (Fig. 1). It was also observed that the difference in population between other treatments and Si treatment for diatoms and rotifers were nearly 3 times while it was lesser for the other three classes. The next highest density of plankton belonging to Trebouxiophyceae was recorded in Mg treatment. The density of plankton belonging to Euglenophyceae

Table 1. Analysis data of soil samples collected before the start of the experiment and cow dung sample used for manuring

Treatment	pH	EC (dS m^{-1})	Organic C (g kg^{-1})	Avl. N (ppm)	Avl. P (ppm)	Avl. K (ppm)	Exch. Ca (ppm)	Exch. Mg (ppm)	Avl. S (ppm)	DTPA[1]-Fe (ppm)	DTPA-Mn (ppm)	DTPA-Zn (ppm)	DTPA-Cu (ppm)	CCSi[2] (ppm)	AASi[3] (ppm)
Control	7.29	0.41	4.0	73.2	8.53	197.5	2010.5	695.0	68.5	4.6	18.5	1.00	2.81	67.73	164.62
Mn	7.29	0.39	2.9	78.9	7.37	182.5	1885.5	697.0	57.2	3.3	23.7	1.02	2.73	-	-
Mg	7.27	0.37	4.1	73.2	7.15	177.5	1943.5	695.0	38.8	3.9	18.9	0.88	2.66	-	-
Si	7.30	0.47	5.0	77.7	7.18	165.0	2004.0	698.0	79.3	6.7	22.4	1.30	2.90	-	-
Zn	7.26	0.41	4.8	78.0	6.69	172.5	1951.5	735.0	67.9	2.8	23.9	0.89	2.48	-	-
Cow dung	-	-	-	2.12%	0.60%	0.33%	2.23%	1.11%	0.07%	825.5 ppm	143.4 ppm	63.8 ppm	11.50 ppm	-	-

[1] Diethylene triamine pentaacetic acid extractable
[2] Calcium chloride extractable
[3] Acetic acid extractable

Table 2. Analysis data of soil samples collected after the experiment

Treatment	pH (1:2.5 ratio)	EC (dS m^{-1})	Organic C (g kg^{-1})	Avl. N (ppm)	Avl. P (ppm)	Avl. K (ppm)	Exch. Ca (ppm)	Exch. Mg (ppm)	Avl. S (ppm)	DTPA[1]-Fe (ppm)	DTPA-Mn (ppm)	DTPA-Zn (ppm)	DTPA-Cu (ppm)	CCSi[2] (ppm)	AASi[3] (ppm)
Control	7.18	0.40	3.7	80.7	1.58	163.7	2965.5	598.0	159.3	8.86	20.2	1.03	3.11	41.82	153.46
Mn	7.05	0.65	3.3	73.5	1.79	220.0	3005.5	656.5	197.4	10.55	41.7	1.93	3.90	44.38	169.25
Mg	7.25	0.39	4.3	90.4	1.52	173.7	3417.7	632.2	131.6	8.46	25.5	1.25	3.50	66.05	191.37
Si	7.22	0.38	5.5	109.9	1.81	146.2	3500.5	622.0	144.5	8.90	21.1	1.73	2.94	43.67	178.12
Zn	7.04	0.51	3.0	78.6	1.50	160.0	3466.0	633.0	194.9	8.20	11.9	2.68	3.03	63.97	191.37

[1] Diethylene triamine pentaacetic acid extractable
[2] Calcium chloride extractable silicon
[3] Acetic acid extractable silicon

Table 3. Water quality parameters (mean ± SD) recorded in the tanks during the experiment

	Control	Mn	Mg	Si	Zn
Temperature (°C)	22.13 ± 1.27	22.05 ± 1.30	22.13 ± 1.35	22.20 ± 1.36	22.25 ± 1.35
pH	8.48 ± 0.18	8.60 ± 0.34	8.45 ± 0.17	8.55 ± 0.23	8.54 ± 0.13
Dissolved oxygen (mg l^{-1})	5.56 ± 1.27	5.86 ± 1.25	5.41 ± 0.63	4.87 ± 0.52	6.46 ± 0.94
Total alkalinity (mg l^{-1})	421.55 ± 30.30	386.77 ± 26.28	434.33 ± 15.76	411.74 ± 18.31	439.08 ± 15.99
Hardness (mg l^{-1})	276.16 ± 27.27	242.33 ± 41.64	266.78 ± 33.02	266.66 ± 34.01	256.16 ± 30.80
Ammonia (μg l^{-1})	105.02 ± 28.69	74.48 ± 10.16	76.30 ± 15.11	80.06 ± 11.20	111.76 ± 28.22
Nitrite (μg l^{-1})	21.73 ± 6.16	19.66 ± 3.78	19.76 ± 4.65	22.43 ± 5.40	17.16 ± 2.88
Nitrate (μg l^{-1})	906.80 ± 306.42	886.86 ± 277.30	857.83 ± 295.49	857.73 ± 282.97	794.06 ± 242.93
Phosphate (μg l^{-1})	224.86 ± 57.40	134.83 ± 86.71	160.80 ± 5 2.41	206.93 ± 63.84	97.26 ± 70.96

was higher in tanks supplemented with Mn, Mg and Si compared to control and Zn treatments. The highest density of Cyanophyceae was recorded in Zn treatment. The density of planktonic crustaceans was higher in control and Mn treatments. In addition to the classes shown in Fig. 1, density of Conjugatophyceae (not included in the Figure) which was represented by two species, *Closterium* sp. and *Spirogyra* sp., was 59.42 (control), 29.71 (Mn), 32.76 (Mg), 21.33 (Si) and 48 no. ml^{-1} (Zn). The total plankton biomass recorded during the experimental period in different treatment tanks indicated lowest values in the 1st month and highest values in the 2nd month (Fig. 2). Except the Zn treatment which recorded a lower value, the biomass of plankton estimated during the experimental period in different treatment tanks did not differ significantly.

The phytoplankton species observed during the present study belonged mainly to five major groups, viz., Bacillariophyceae, Coscinodiscophyceae, Chlorophyceae, Cyanophyceae and Euglenophyceae, which reflected the usual phytoplankton composition of tropical fish ponds (Gangadhar et al., 2018). Growth of phytoplankton is a function of (1) the availability of major nutrients and micro-nutrients; (2) physical parameters such as light, temperature and osmotic pressure; (3) trace organic substances which may be inhibitory or stimulatory; and (4) the genetic potential for growth inherent in the phytoplankton (Sousoni, 2018). In the present study, no major difference was recorded between the different treatment tanks with respect to water quality and all the parameters were within the acceptable range for carp seed rearing (Jena et al., 2011). Similar conditions were maintained for all the treatment groups except for the differences in the additional nutrients supplemented. Hence, the differences in the taxonomic composition of plankton between treatments can be attributed to the nutrients supplemented. According to Sunda (1989), wide differences exist among algal species in their requirements for nutrient metals and thus the predominant effect of trace metals may well be on the species composition of phytoplankton communities.

Irrespective of the treatments, the density of diatoms (Bacillariophyceae and Coscinodiscophyceae) was the highest among all other classes of plankton encountered. According to Boyd (2014), diatoms prefer nitrate over ammonium as the nitrogen source and ammonium is the preferred nitrogen source of green and blue-green algae. In the present study, the nitrate level in culture water was higher than ammonium and the population of diatoms was the highest among all the planktonic groups irrespective of the treatments, indicating a suitable environment for diatom growth. Further, alkaline water is known to favour the abundance of diatoms (Kumar & Oommen, 2009). In the present study, the pH of culture water was alkaline, ranging from 8.45 to 8.60, favouring the growth of diatoms.

The density of diatoms (Bacillariophyceae and Coscinodiscophyceae) and rotifers was nearly three times higher in tanks supplemented with Si than other treatments. Diatoms are the usual food for zooplankton and filter feeding fishes. As diatoms have good nutritional value and do not degrade water quality, shrimp farmers often attempt to

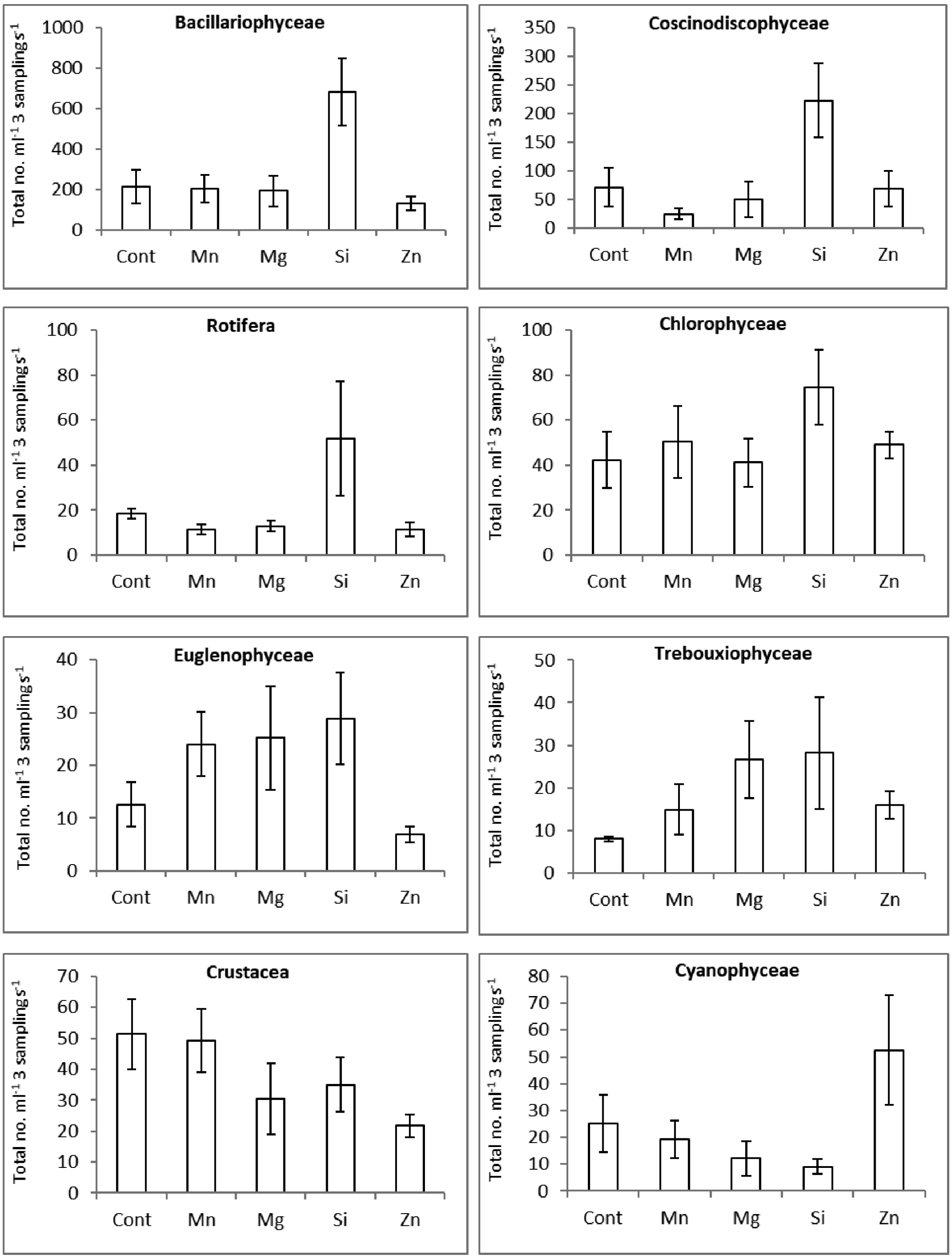

Fig. 1. Total density (Total no. ml^{-1} 3 samplings^{-1}) of planktonic classes

increase diatoms instead of other planktonic algae. Frey & Small (1980) reported that while major nutrients limit the growth of communities, the outcome of inter-generic competition for these nutrients to a large extent is under the control of micronutrients leading to the dominance of a few phytoplankton genera. Diatom production is dependent on the availability of dissolved silicic acid [$Si(OH)_4$] and sub-optimal silicon availability affects the rate of diatom biogenic silica ($bSiO_2$) production and can limit their growth (Krause et al., 2018). In diatom cells, the ratio of carbon: nitrogen: silicon:

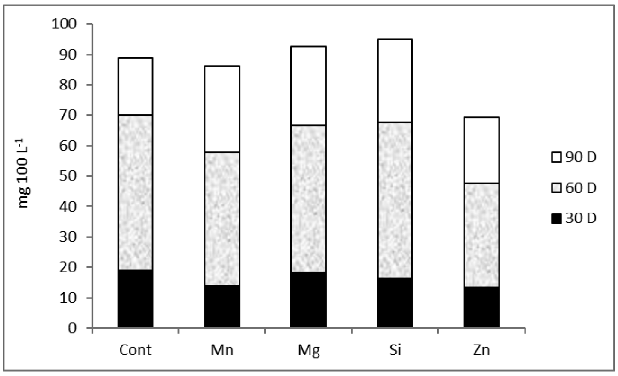

Fig. 2. Biomass (AFDM; average; mg 100 L^{-1}) of plankton recorded during the experimental period in different treatment tanks

phosphorus is around 106:15:16:1. Thus, diatoms require about the same amounts of nitrogen and silicon for growth (Boyd, 2014). Si transporters, the specific membrane-associated proteins shown to transport $Si(OH)_4$ across lipid bilayer membranes (Hildebrand et al., 1997) have been identified in numerous diatom species (Thamatrakoln et al., 2006). Diatom cells typically reach a maximum uptake rate when silicic acid concentrations exceed 0.2–8 μmol L^{-1}, depending on the diatom species (Martin-Jezequel et al., 2000). At concentrations below these levels, the uptake rate is reduced, and the rate of incorporation of silicic acid into the frustule cannot keep up with the rate of cell division (Harrison et al., 1977). In addition to this mode of uptake, diffusion of silicic acid into the cell also happens at high silicic acid concentrations (>20 μmol L^{-1}) (Finkel et al., 2010). The organic nitrogen and phosphorus components of the diatoms will be recycled rapidly back to inorganic form through zooplankton grazing. However, the dissolution and recycling of silicon is at a much slower rate than the organic nitrogen and phosphorus components. Hence, it is rational to expect a continuous supply of bio-available forms of silicon for the sustained growth of diatom in a culture system having optimal Si levels in the beginning.

The relationship between silicon and diatom bloom has been studied in marine environments (Harrison et al., 1977; Krause et al., 2018). Experiments in which samples of Sargasso Sea surface water were enriched with different nutrients showed that enrichment with N, P and Si stimulated the growth of several species of diatoms (Menzel et al., 1963). Saraswathy et al. (2012) reported that phytoplankton density had a positive correlation with the silicate content of pond water in addition to nitrate and phosphate. An increase in silicate increases the proportion of diatoms as compared to green algae in the phytoplankton communities (Kilham, 1986). Boyd (2014) observed that if the silica level in seawater is less than the 6.4 mg SiO_2 L^{-1}, applying sodium silicate to sea water containing 0.21 mg L^{-1} silica was quite effective in increasing the abundance of diatoms.

Kilham (1971) revealed the relationship between ambient silica concentrations and the dominance of specific freshwater diatoms. Silicon can be provided by applying sodium silicate or calcium silicate, but silicon from these sources would not be highly soluble in waters of pH <9 (Boyd, 2000). In the present study, we have used ortho-silicic acid $[Si(OH_4)]$, the fundamental building block of bio-silicas which is water soluble and stable in highly diluted aqueous solutions as the source of Si for phytoplankton. Silicic acid is the bio-available form of silicon which can be assimilated by diatoms via endocytosis in the form of partially condensed silicic acid through the surface (Annenkov et al., 2020). The density of planktonic Cyanophyceae was the least in Si treatment. The presence of bio-available silicon resulted in plankton population rich in diatoms (Bacillariophyceae and Coscinodiscophyceae) and poor in planktonic Cyanophyceae. It has been reported that maintenance of a healthy diatom population in the ponds will keep undesirable blue-green algae in check (Vuuren et al., 2006). A relationship between the availability of silicon and the population of diatoms and planktonic Cyanophyceae has been recorded by Arai & Fukushima (2014) and Schelske & Stoermer (1971 & 1972). Cyanobacteria are usually favoured in an environment of low nitrogen availability, but diatoms usually dominate in nutrient-rich conditions (Tilman et al., 1986). Diatoms consume nutrients, N and P, reducing their availability to other phytoplanktons, Cyanophyceae and Conjugatophyceae. Diatoms are considered desirable in aquaculture ponds because they do not degrade water quality and are of good nutritional value to many aquaculture species. During early post larval development of fish and shrimp, high mortality is observed, particularly due to a shortage of live feed. A relatively large number of fish include diatoms in their menu during the initial period of life. Live diatom is better than feed pellet since it creates a triggering effect by its continuous movement allowing a better perception for feeding larva. The swimming activities of the live food ensure an even distribution in the water column, allowing more frequent encounters with larva (Lavens & Sorgeloos, 1996) and are likely to stimulate larval feeding response (David, 2003).

The dominance of rotifers in Si treatment suggests that this zooplankton community could utilize the newly grown diatoms rapidly. Thus, the nutrient addition induced the growth of selected species (diatoms) that are susceptible to zooplankton grazing (Kilham, 1980). An increase or decrease in the abundance of diatoms was followed by a subsequent increase or decrease in the rotifer population (Roy, 2014). Microalgae like diatoms are not only live feed for rotifers but also stimulate enzymatic synthesis and onset of feeding in young fish. Rotifers are one of the two dominant zooplankton prey preferred by shrimp and fish, copepods being the other group.

Chlorophytes are also among the important algae consumed by phytoplankton-feeding fish (Ludwig et al., 1998). Though the population density of Chlorophytes was higher under Si treatment compared to other treatments, its overall population was lower (<100 ml^{-1}) compared to that of diatoms (>100 ml^{-1}) irrespective of the treatment. The next higher density of phytoplankton belonging to Euglenophyceae and Trebouxiophyceae (a class of green algae, in the division Chlorophyta) was recorded with Mg treatment. Studies by Siddique et al. (2012) have shown that Euglenophyceae members are consumed by carps like catla, mrigal, silver carp and big head carp. Lu et al. (2004) also reported that larval tilapia, *Oreochromis niloticus* ingested significantly less *Euglena gracilis* but the ingestion was higher than the *Chlorella vulgaris*. Euglena blooms were not found to be harmful to fish growth, rather they were found to contribute oxygen to the environment by the process of photosynthesis (Brunson et al., 1994).

Studies by Peers & Price (2004) demonstrated that Mn is also a limiting factor in the growth of marine diatoms *Thalassiosira pseudonana* and *T. oceanica*. The results of the study conducted by Pausche et al. (2019) demonstrated that low Mn availability inhibits the growth of Antarctic diatom, *Chaetoceros debilis.* However, in our study, the density of diatoms was lower under Mn treatment than S and was comparable with that of the control.

Clear evidence of Zn limitation of phytoplankton production was difficult to obtain (Baines et al., 2016). However, a positive relationship between Zn concentration and plankton species has been reported (Coale et al., 2003). Adhikari & Ayyappan (2004) determined the role of zinc on plankton productivity. In our study, under Zn treatment, the density of planktonic Cyanophyceae was the highest and that of Crustacean zooplankton like copepods, daphnia and nauplius was the least. Cyanobacteria are a poor food source for freshwater grazers, when compared to small Chlorophyceae and/or flagellates due to poor digestibility by fish (Stockner et al., 2000). Evidence suggests that Cyanobacteria are low-quality food for Copepods owing to their protease inhibitors (Ruokolainen et al., 2006).

In our study, it was observed that the addition of nutrients did not alter the total planktonic biomass significantly. There are conflicting reports on micronutrient limitation of the final biomass yield of phytoplankton. The addition of iron resulted in an increased growth rate but not the biomass of phytoplankton genera (Menzel et al., 1963). Contrary to this observation, Buzancic et al. (2016) recorded that an increase in the concentrations of nitrates, nitrites, ammonium salts and ortho-silicates had a positive effect on the biomass of phytoplankton in the Mali Ston and Kaštela Bay area. Frey & Small (1980) opined that while micro-nutrients have marked effects on the species composition, major nutrients have substantial effect on final yield of plankton. The insignificant differences in the planktonic biomass in the present study may be attributable to the similar supplementation level of major nutrients for all the treatments.

The study revealed that the supplementation of nutrients to the culture system affects the taxonomic quality of plankton produced. Enrichment with a bio-available form of Si leads to a desirable plankton population consisting mainly of diatoms and rotifers, a preferred food for fish larvae. The results of the present study will have implications for the enhancement of live food organisms in general and diatoms in particular in the culture environment. However, further studies on evaluating the effect of varied levels of incorporation of silicic acid on the taxonomic composition of plankton and the growth of freshwater fish are warranted.

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