



# In vivo statistical evaluation of the efficacy of a biological agent on water quality in the rearing of ornamental *Cyprinus carpio*

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## INTRODUCTION

A common disease affecting ornamental *Cyprinus carpio* (koi) around the world is known as "springtime", or ulcer disease. Several conditions prevalent at this time of the year, such as parasitic infestations, and dynamic changes in the environment can result in severe attenuation of the immune response of the fish due to drastically changing water temperatures, and a multitude of foreign infections. Pathogens such as *Aeromonas spp.* and *Pseudomonas spp.* infect weakened fish resulting in diseases such as haemorrhagic septicaemia, ulcerative erythrodermatitis, dropsy and abdominal oedema.

Strategies focused on reducing pathogenic microbial load and the concentration of waste metabolites such as ammonium, nitrate, nitrite and phosphate are an essential component of ornamental carp husbandry. Chemical treatments are an option but are costly, environmentally harmful and result in enhanced resistance of pathogenic organisms. Biological treatment is an emerging alternative because it offers potential for the reduction in the concentration of pathogenic bacterial species and waste metabolites, thus resulting in a holistic improvement in fish health.

A biological treatment, containing consortia of *Bacillus spp.*, isolated from natural environments in Gauteng, South Africa, was developed based on *in vitro* studies. These studies demonstrated that the isolates had higher growth rates than the and possessed the ability to reduce waste metabolite concentrations. These isolates were formulated into a product prototype, which was subsequently evaluated in *in vivo* trials at 100 L scale.

## OBJECTIVES

The objective of this investigation was to demonstrate the enhancement of water quality by administration of a prototype product containing isolates of *Bacillus spp.*

## MATERIALS AND METHODS

The trials were conducted at the CSIR in six aquaria. Each aquarium contained 100 l of water with identical aeration, temperature and filtration systems. The aquaria were allowed to acclimatize for one week prior to the introduction of fish. Each of the control aquaria were stocked with 39.2g ± 1.2g juvenile *Cyprinus carpio* and the test tanks stocked with 37.6g ± 1.2g. Three aquaria were dosed with the biological agent (test) and the remaining three supplemented with a placebo (control). Treatments were administered once a week, alternating each week between two different consortia of *Bacilli*. The trial duration was ~ 80 days. At termination of the trial each aquarium was mixed to re-suspend all filter material and solids. Samples were extracted and analyzed for the concentration of *Bacillus spp.*, *Aeromonas spp.* and *Pseudomonas spp.* by plate count assays (CFU). Nitrate, Nitrite and Phosphate concentrations were analyzed by ion chromatography, Ammonium concentration was measured using a Merck Reflectoquant instrument. Turbidity and chlorophyll concentrations were also measured. All the results obtained were subjected to comparative statistical analyses using the students T-test.

Results obtained for analysis of key variables from each group of aquaria (test and control) are presented in Table 1. Treatment with the biological agent resulted in improved water quality in comparison to the untreated control. Figures 1 to 7 provide graphical indications of differences in key water quality variables.

## RESULTS AND DISCUSSION

Results obtained for analysis of key variables from each group of aquaria (test and control) are presented in Table 1. Treatment with the biological agent resulted in improved water quality in comparison to the untreated control. Figures 1 to 7 provide graphical indications of differences in key water quality variables.

- Reduced pathogen counts in aquarium water result in a reduced incidence of pathogenic infections. Total pathogen counts were found to be 85 % lower in the test aquaria ( $p < 0.05$ ) compared to the control.
- High concentration of Ammonium and Nitrate ions can be toxic to fish therefore facilitating the reduction of these ions would improve fish health and water quality. The test aquaria had significantly lower ( $p < 0.05$ ) concentrations of these ions in comparison to the control.
- Algae rely on many micro nutrients for their growth, nitrate and phosphate being two

of the most important. Reduced nitrate and phosphate concentrations in the test treatment resulted in reduced algal growth. This is substantiated by a significantly lower concentration of chlorophyll ( $p < 0.05$ ) in the test treatments in comparison to the control treatments.

- Turbidity is an indication of water clarity, which is a key component of pond husbandry. Results obtained indicated that the test aquaria had significantly lower turbidity measurements than the control ( $p < 0.05$ ).

Analysis	Units	Test			Control			P value
		Average	SD	CV	Average	SD	CV	
Bacillus Counts	Cells/mL	1.00E+04	1.15E+03	11.5	3.03E+03	9.36E+02	30.9	2.37E-09
Pathogen Counts	Cells/mL	1.72E+04	3.78E+03	22.0	1.19E+05	2.97E+04	25.0	1.65E-06
Turbidity	NTU	0.1	0.0	5.7	0.2	0.0	17.3	1.10E-03
Chlorophyll	mg/L	0.03	0.01	19.5	0.04	0.01	16.2	1.74E-02
Nitrate	mg/L	54.4	6.2	11.4	68.4	4.2	6.2	4.21E-05
Nitrite	mg/L	ND	ND	ND	ND	ND	ND	-
Phosphate	mg/L	8.4	1.6	19.4	14.8	1.3	9.0	3.10E-05
Ammonia	mg/L	0.35	0.07	20.2	0.63	0.05	7.4	6.76E-04

Table 1

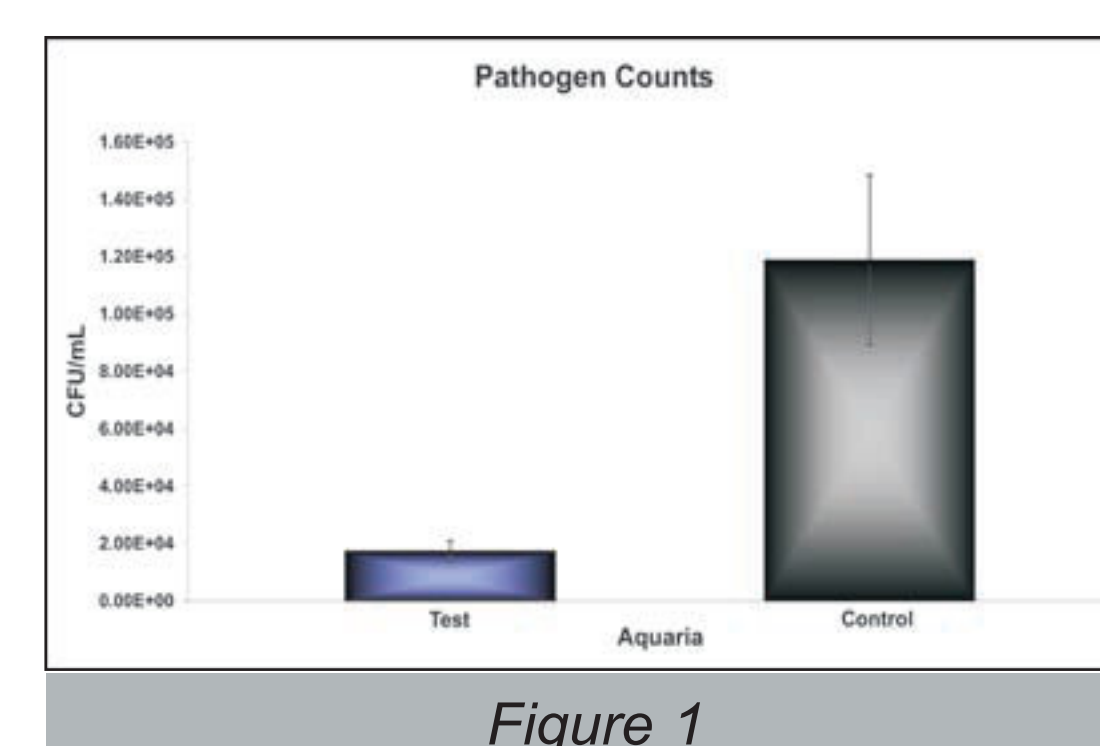
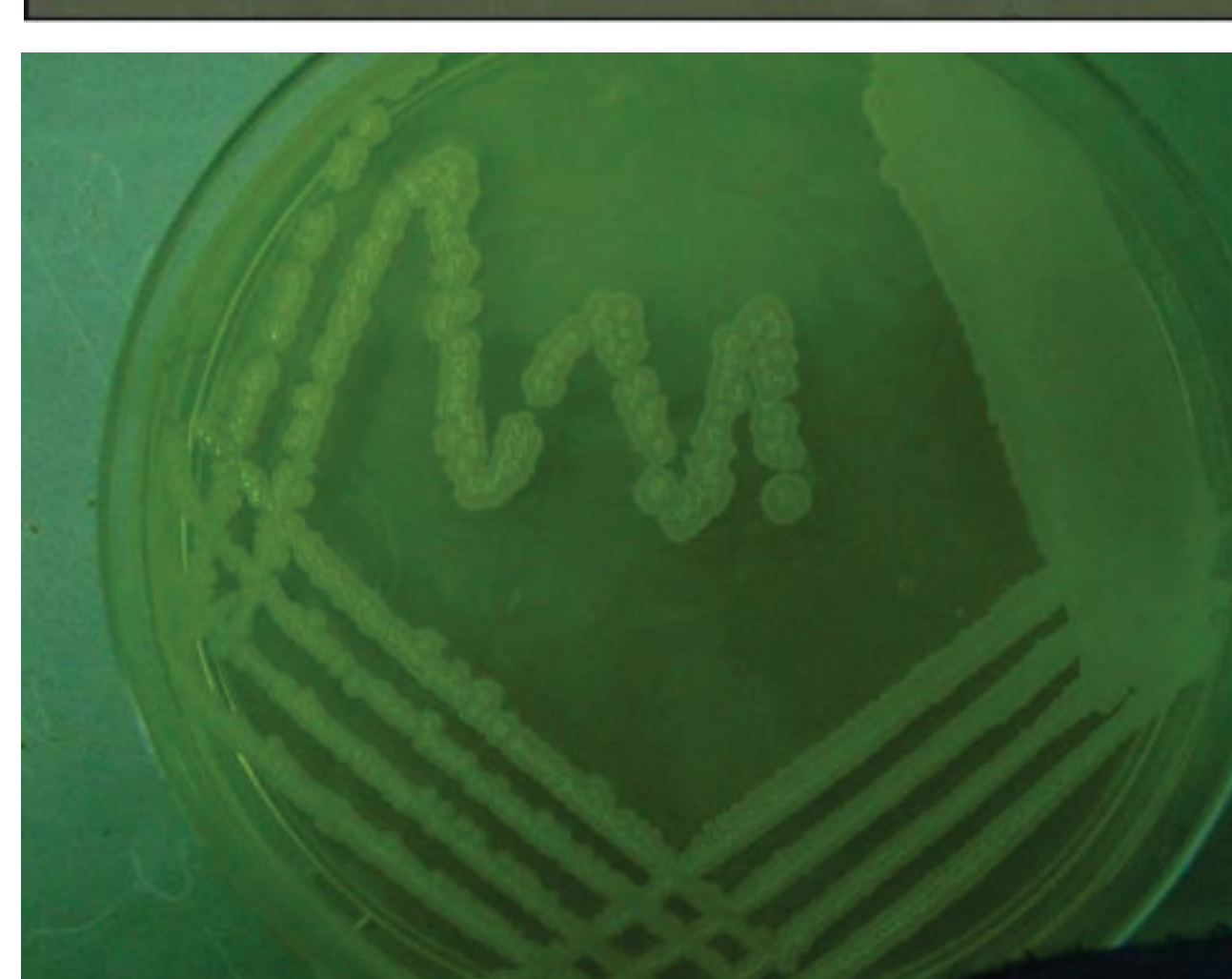


Figure 1

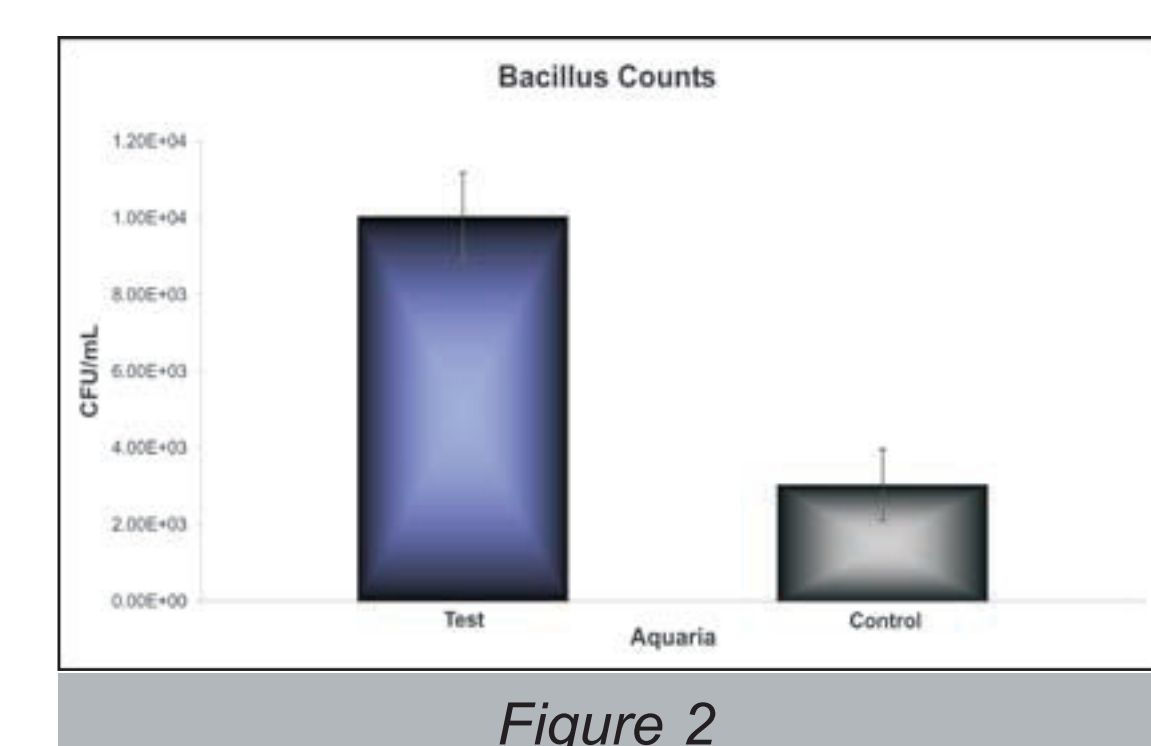


Figure 2

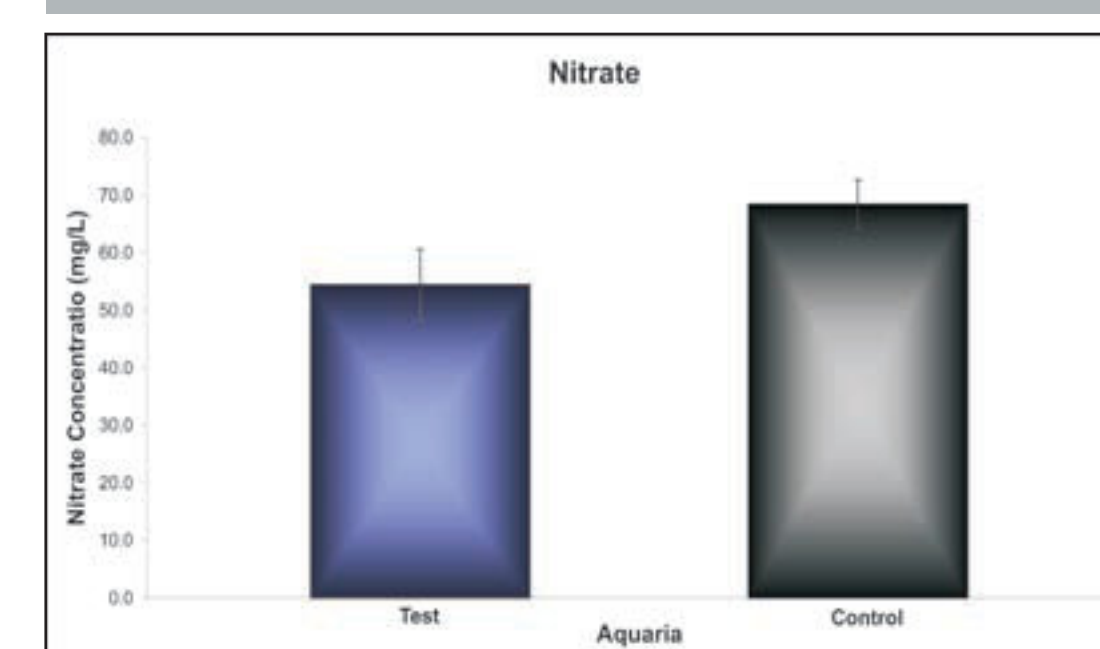


Figure 3

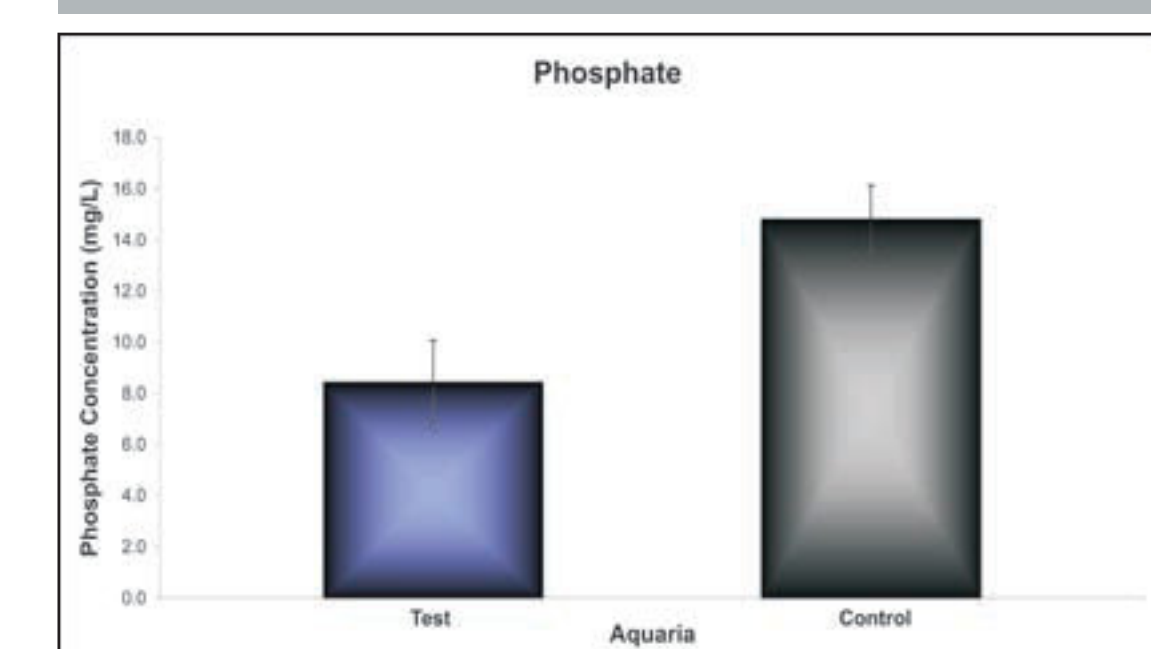


Figure 4

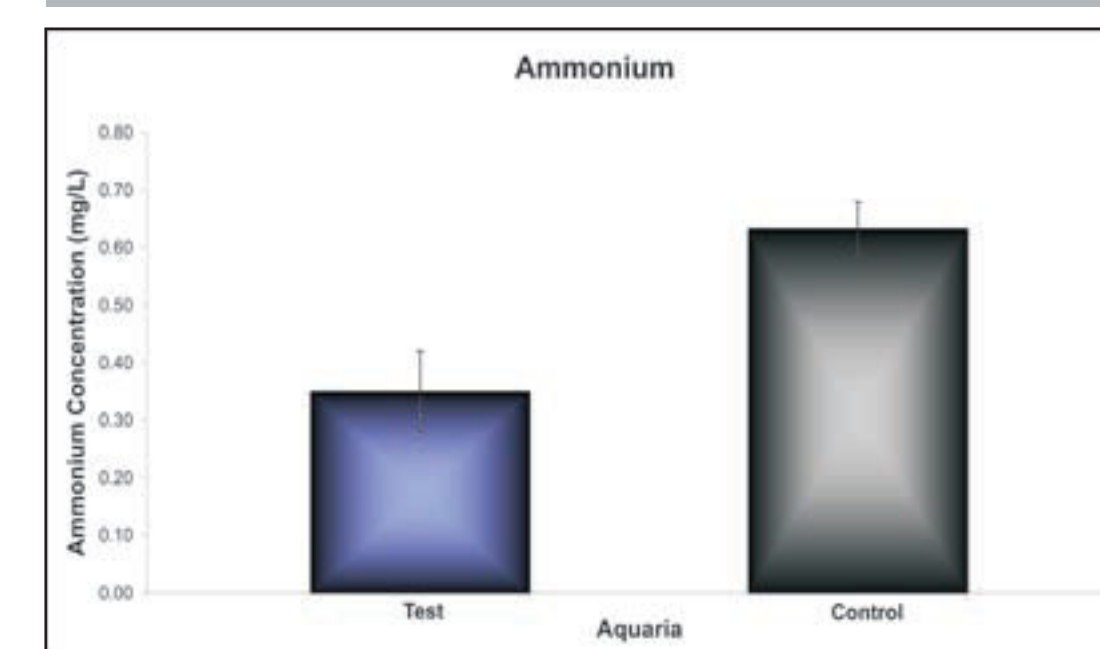


Figure 5

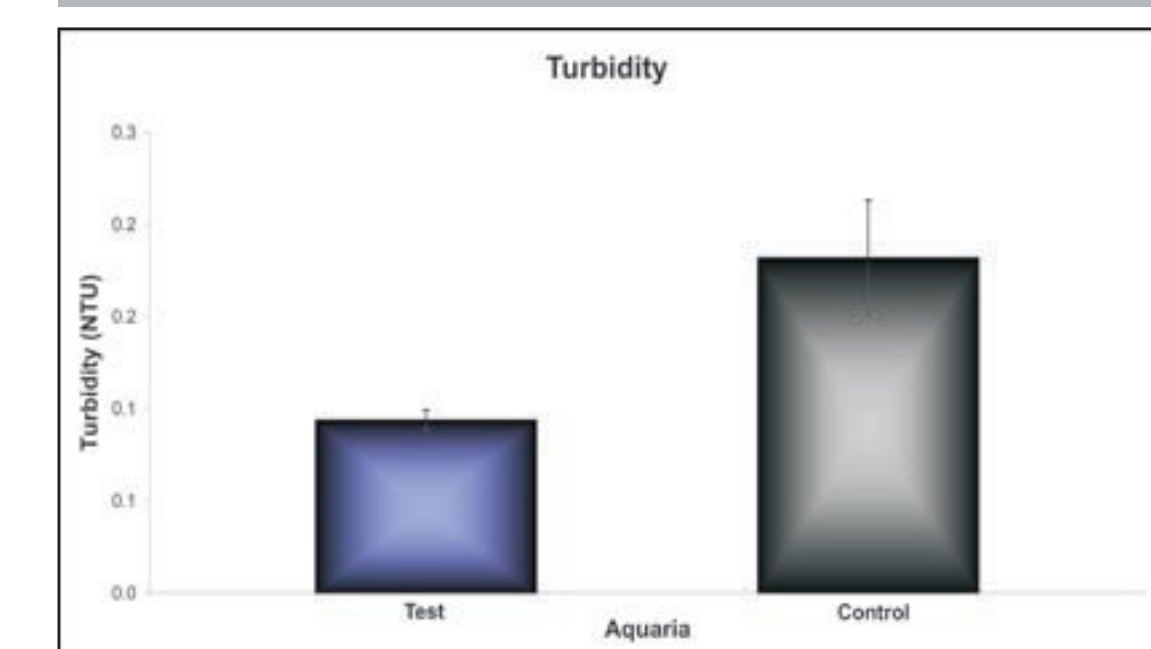


Figure 6

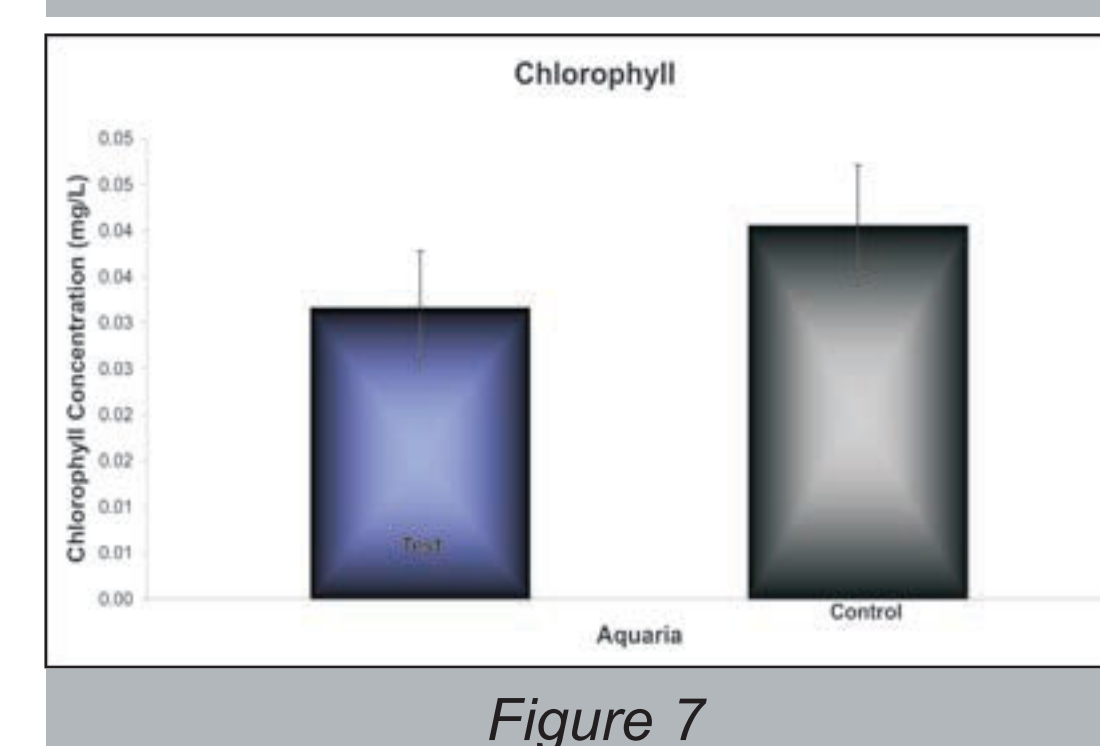


Figure 7

## CONCLUSION

The results obtained from the *in vivo* trials indicate that there is a significant improvement in water quality when the model systems were treated with the biological agent. The consortia of *Bacillus spp.* demonstrate potential as a commercial product for enhancement of water quality in ornamental *Cyprinus carpio* ponds.

## ACKNOWLEDGEMENTS

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