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Study on the Reuse of Treated Wastewater on the Behavior of Aquatic Biodiversity: Case of *Anodonta cygnea* and *Cyprinus carpio* from Western Algeria

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ABSTRACT

Water scarcity in semi-arid regions necessitates innovative strategies for sustainable resource management. This study evaluates the biofiltration capacity of the freshwater mussel *Anodontacygnea* and the physiological tolerance of the common carp fry *Cyprinus carpio* when exposed to treated wastewater. Experimental trials using effluents from the Sidi Bel Abbès activated sludge treatment plant revealed that higher mussel densities markedly enhanced water quality by lowering turbidity, suspended solids, biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), and nutrient loads (nitrogen and phosphorus), while concurrently improving dissolved oxygen. In parallel, *C. carpio* fry exposed to increasing effluent concentrations (up to 100%) exhibited high survival and resilience, even under stressful conditions characterized by low oxygen availability and elevated ammonium and nitrite levels. These findings underscore the dual potential of mussel biofiltration and resilient aquaculture species in promoting the sustainable reuse of treated wastewater offering a viable ecological pathway for aquaculture development in water-limited environments.

INTRODUCTION

The reuse of treated wastewater has become a critical strategic priority in arid and semi-arid regions, where the increasing scarcity of water resources demands integrated and sustainable management approaches **(Angelakis & Snyder, 2015)**. In Algeria, this challenge is further intensified by the combined effects of climate change and the growing agricultural and industrial demands, thereby highlighting the importance of reusing treated effluents **(Boudjellal *et al*., 2021)**.

Wastewater treatment plants (WWTPs) play a central role in this context by reducing organic and nutrient loads prior to discharge or reuse. However, the actual efficiency of these facilities depends not only on the physico-chemical and biological processes applied but also on the final effluent quality and its potential impact on receiving aquatic ecosystems **(Tchobanoglous *et al*., 2014)**.

The incorporation of biofiltering organisms such as *Anodonta cygnea* into post-treatment systems represents a natural, sustainable, and cost-effective solution to further improve water quality, thereby complementing conventional processes **(Karatayev *et al*., 2015)**. Moreover, assessing the tolerance of fish species such as the common carp *Cyprinus carpio*, widely used in aquaculture, provides valuable indicators of the compatibility of treated water with aquatic biodiversity and its potential for reuse **(FAO, 2020)**.

This study aims to assess the performance of the Sidi Bel Abbès WWTP and to examine the complementary role of *A. cygnea* in enhancing the physico-chemical and biological quality of treated wastewater, while evaluating the tolerance of *C. carpio* fry to varying proportions of treated effluents.

MATERIALS AND METHODS

1. Study area

This research was conducted in western Algeria, focusing on three sites of hydrological and ecological relevance. The present study was conducted at Lake Sidi Mohamed Benali at 35°14′38.6″ N latitude and 0°38′50.6″ W longitude. The site lies within the commune of Aïn Trid, approximately 1.7km from the city of Sidi Bel Abbès, and represents one of the main freshwater reservoirs in the region. This artificial lake plays a pivotal role in local hydrological regulation and sustains a rich and diverse aquatic biodiversity.

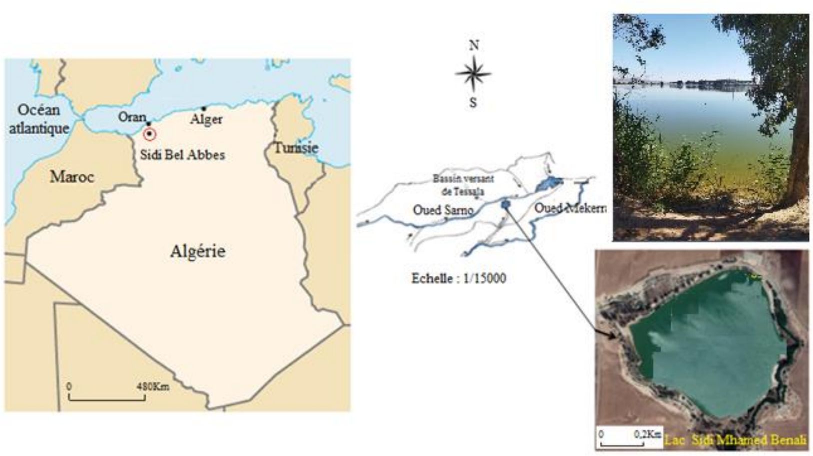


Fig. 1. Lake of Sidi Mohamed Benali

The Sidi Bel Abbès wastewater treatment plant (WWTP) is located northeast of the city, at 35°13′24.53″ N latitude and 0°36′24.46″ W longitude. The facility employs combined biological and physico-chemical processes to treat domestic wastewater prior to discharge or potential reuse. The overall performance of this WWTP plays a critical role in determining the quality of the final effluent and its potential impact on downstream aquatic ecosystems.

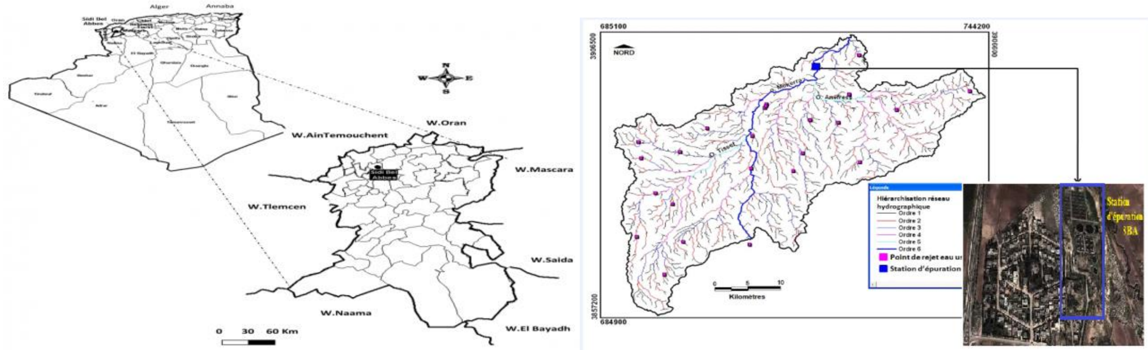


Fig. 2. Sidi Bel Abbès wastewater treatment plant (WWTP)

The study area is precisely situated at 33°09′14.10″ N latitude and 1°16′08.17″ E longitude, within the commune of Brezina, approximately 80km south of El Bayadh. This region represents an important agro-ecological zone, where water from the Brezina Dam is predominantly utilized for the irrigation of palm groves and surrounding agricultural lands.

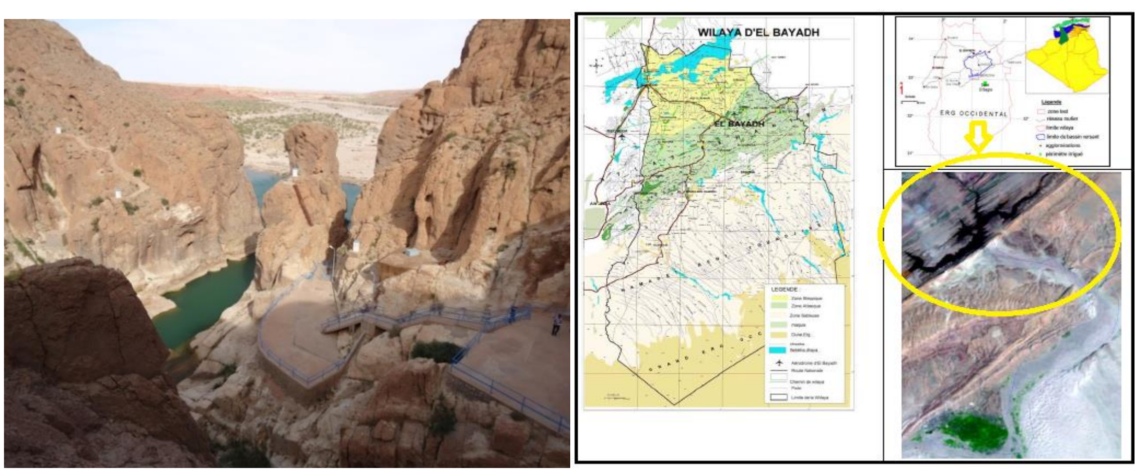


Fig. 3. Brezina Dam

2. Biological material

In January 2017, twenty-five freshwater mussels *Anodonta cygnea* were randomly sampled from the Brezina Dam and were transported alive to the laboratory.

Fig. 4. *Anodonta cygnea*

In February 2017, thirty fry of the common carp *Cyprinus carpio* were collected from Sidi Mohamed Benali Lake using a plankton net. The fish were transported alive and acclimatized under controlled laboratory conditions prior to experimentation.

Fig. 5. Fry of *Cyprinus carpio*

3. Wastewater sampling and analysis

From January to May 2017, physico-chemical analyses were conducted at the Sidi Bel Abbès WWTP. Two sampling points were considered:

- Raw wastewater (RW): collected immediately after screening.
- Treated wastewater (TW): collected at the clarifier outlet.

Samples (500 mL) were collected in polyethylene bottles, stored on ice, and analyzed in the WWTP laboratory following the **APHA (2012)** standard methods.

4. Experimental design

4.1. Filtration by Anodonta cygnea

Filtration experiments were carried out under controlled laboratory conditions using pretreated domestic wastewater (Fig. 6a). Beakers (2–3 L) were used with four

mussel density treatments:
- M1: 3 individuals (low density)
- M2: 5 individuals (medium density)
- M3: 6 individuals (medium-high density)
- M4: 10 individuals (high density)

A negative control (NC), consisting of treated wastewater without mussels, was included. All treatments were maintained under identical conditions to assess filtration efficiency relative to mussel density.

4.2.Tolerance of Cyprinus carpio

The tolerance of carp fry was tested under four treatments:
- M1 (control): lake water (0%)
- M2: 25% treated wastewater
- M3: 50% treated wastewater
- M4: 100% treated wastewater (Fig. 6b)

Experimental conditions were maintained as follows: temperature 22– 25°C; photoperiod 12h light/ 12h dark; continuous aeration; 50% water renewal every three days; and feeding twice daily (2–3% body weight). The trial lasted approximately for four months, with monitoring conducted at four sampling dates (S1–S4).

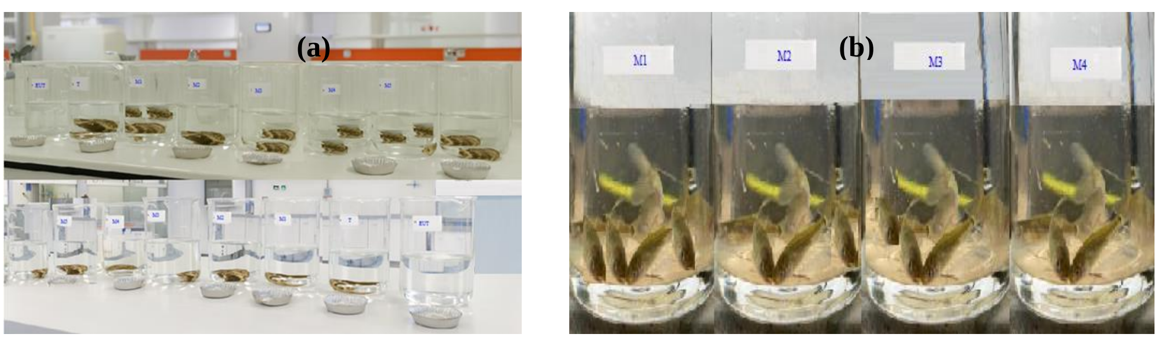


Fig. 6. Beakers with M1–M4 solutions under mussel density and treated wastewater concentration treatments: (a). Experimental setup of *Anodonta cygnea*; (b). Experimental setup of *Cyprinus carpio* .

5. Monitored parameters

- Physico-chemical parameters, including temperature, pH, conductivity, turbidity, dissolved oxygen, total suspended solids (TSS), BOD_5, COD, ammonium (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-), and phosphate (PO_4^{3-}), were tested.

- Biological parameters, including daily survival counts, behavior (activity, feeding, response to stimuli), growth, and mussel filtration performance, were conducted.

6. Statistical analysis

Data were statistically analyzed to explore relationships between water quality and biological responses. Pearson correlation tests were performed to assess associations between fish survival, growth, and behavior, as well as between mussel filtration efficiency and water quality parameters. All statistical analyses were performed using IBM SPSS Statistics software (version 25; IBM Corp., Armonk, NY, USA). Pearson correlation tests were used to evaluate associations between fish survival, growth, behavior, mussel filtration efficiency, and water quality parameters. Statistical significance was set at $P < 0.05$.

RESULTS

The physicochemical monitoring of raw (RW) and treated wastewater (TW) from the Sidi Bel Abbès treatment plant (Table 1) showed clear temporal variations across the five sampling campaigns (S1–S5), reflecting both treatment efficiency and seasonal influences. Temperature The pH remained stable (7.8–8.5) in both RW and TW, confirming effective buffering capacity **(Boyd, 1990)**. Nutrient patterns revealed nitrification in TW, with occasional nitrite peaks in RW (0.33mg/ L at S4) under high organic load. Phosphate fluctuated widely (0.44–5.05 mg/L), with residual levels in TW (< 1.7mg/ L) remaining ecologically relevant for eutrophication **(Smith *et al*., 1999)**. followed a typical Mediterranean pattern, increasing from winter (11– 14°C at S1–S2) to late spring (21°C at S4), before slightly decreasing in May (18°C at S5). This seasonal trend strongly influenced microbial metabolism and organic matter decomposition **(Wetzel, 2001)**.

Table 1. Physicochemical characteristics of domestic wastewater from the Sidi Bel Abbès wastewater treatment plant (2017)

Sampling Date	**S1 : (22/01/2017)**		**S2: (12/02/2017)**		**S3 : 04/03/2017)**		**S4: (23/04/2017)**		**S5 : (16/05/2017)**	
Wastewater type	RW	TW	RW	TW	RW	TW	RW	TW	RW	TW
T°(°C)	13.98	13.21	11.71	11.38	15.25	15.04	21.29	21.33	18.17	18.32
PH	8.53	8.49	8.26	8.29	7.84	7.82	7.92	8.01	8.04	7.99
Conductivity(µs/cm)	1372	1183	1445	1142	1559	1859	1817	1295	1855	1491
Turbidity(NTU)	306	23.87	512	44.72	120	27.06	615	27.82	224	13.02
TSS (mg/l)	253.41	7.60	461.5	32.40	726.5	6.00	1935	12.00	618.5	8.00
BOD_5 (mg/l)	209	15.60	209	10.20	356	16.90	569	17.5	481	26.8
COD (mg/l)	646.13	30.92	529.48	36.69	1201.12	62.47	1054.9	46.4	990.8	49.17
COD/ BOD_5 (mg/l)	2.22	1.98	2.53	3.59	3.37	3.69	0.04	0.06	2.05	1.83

NO_3-N (mg/l)	0.25	1.05	0.01	1.01	0.05	0.09	0.01	0.012	0.06	0.05
NO_2-N (mg/l)	0.01	0.17	0.01	0.08	0.006	0.01	0.33	0.61	0.02	0.01
PO_4^- (mg/l)	0.59	1.70	2.88	1.47	0.44	0.79	5.05	3.70	1.04	0.03

Conductivity ranged from 1150–1450 µS/cm in winter to >1800µS/ cm in spring, reflecting enhanced mineralization and domestic inputs **(Allan & Castillo, 2007)**. Turbidity and TSS reached very high peaks in RW during spring (up to 1935mg/ L at S4), but TW consistently showed >95% removal (<32mg/ L), in line with international standards **(Von Sperling, 2007)**. Organic load indicators (BOD_5, COD) showed similar seasonal enrichment in RW (up to 569mg/ L BOD_5 and 1201mg/ L COD), whereas TW values remained below 27mg/ L (BOD_5) and 62mg/ L (COD). The COD/BOD_5 ratio (2.2–3.6) (Table 1) indicated mixed fractions of easily and slowly degradable matter **(Metcalf & Eddy, 2014)**.

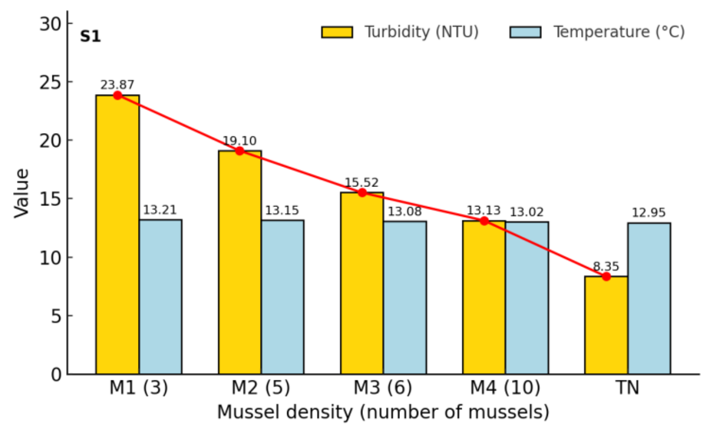
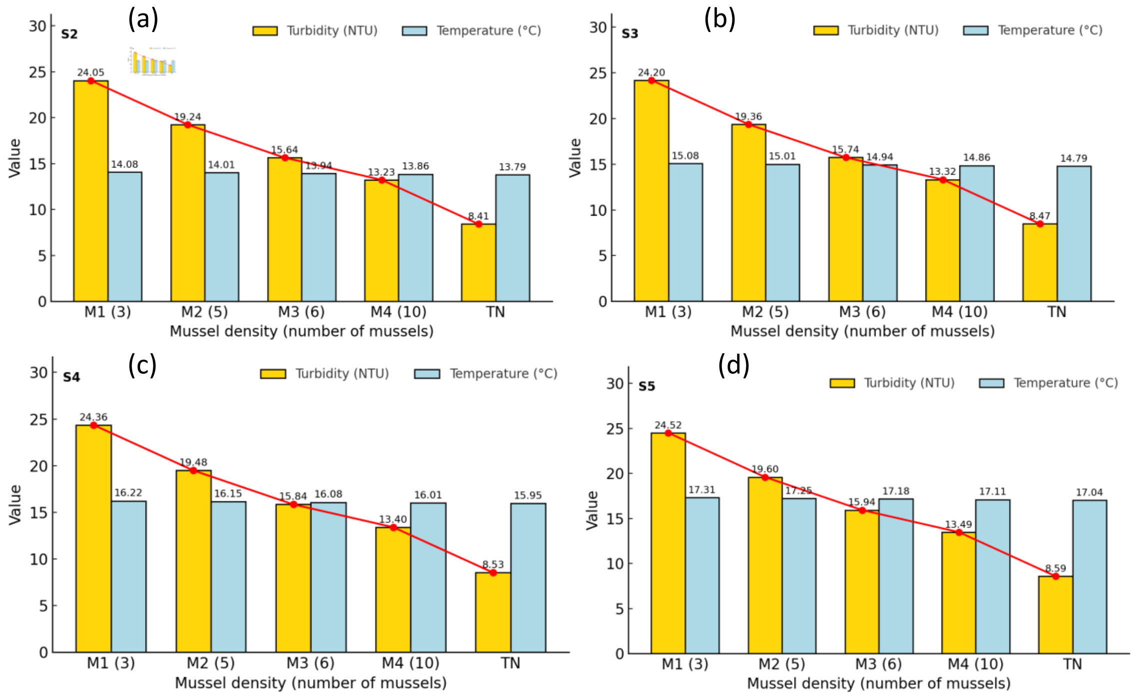


Fig. 7. Parameters of turbidity & temperature at S1 across mussel densities (M1–M4, TN)



(a): S2 (12/02/2017), (b): S3 04/03/2017),
(c): S4(23/04/2017), (d): S5 (16/05/2017)

Fig. 8. Parameters of turbidity & temperature at S2-S5 across mussel densities (M1–M4, TN)

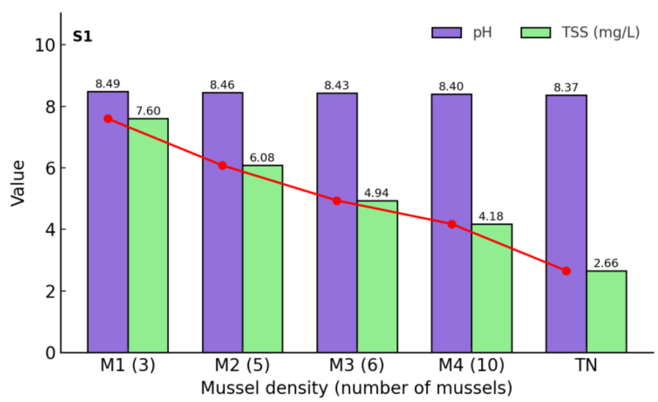
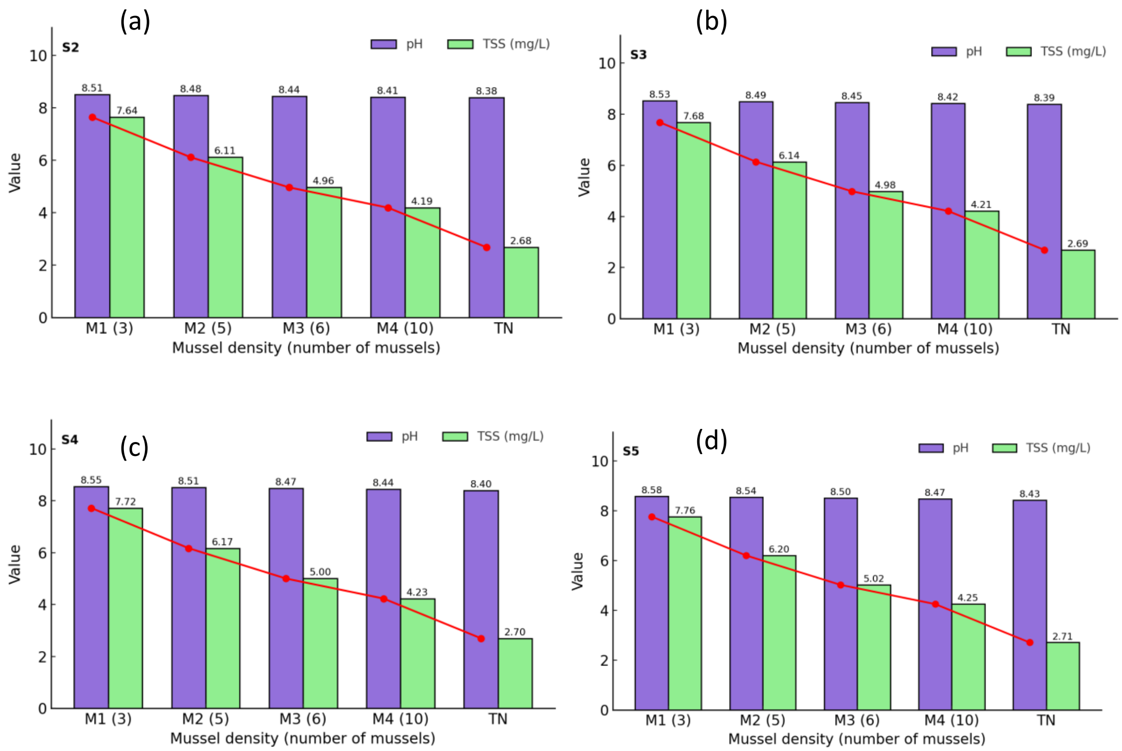


Fig. 9. Parameters of pH & TSS at S1 across mussel densities (M1–M4, TN)



(a): S2 (12/02/2017), (b): S3 04/03/2017),
(c): S4(23/04/2017), (d): S5 (16/05/2017)

Fig. 10. Parameters of pH & TSS at S2-S5 across mussel densities (M1–M4, TN)

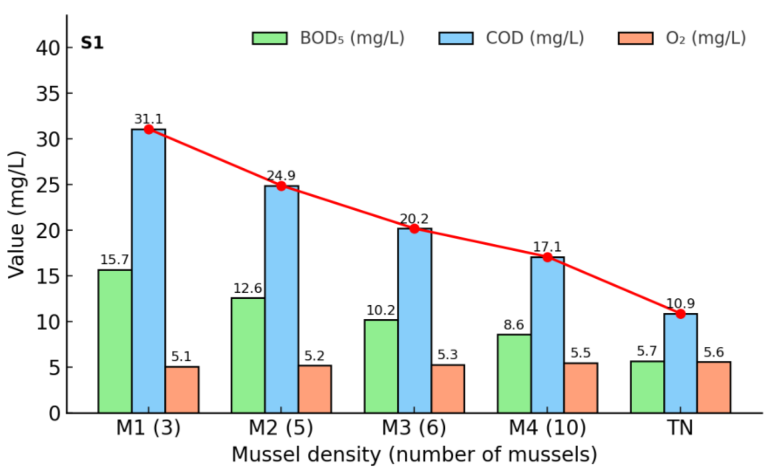
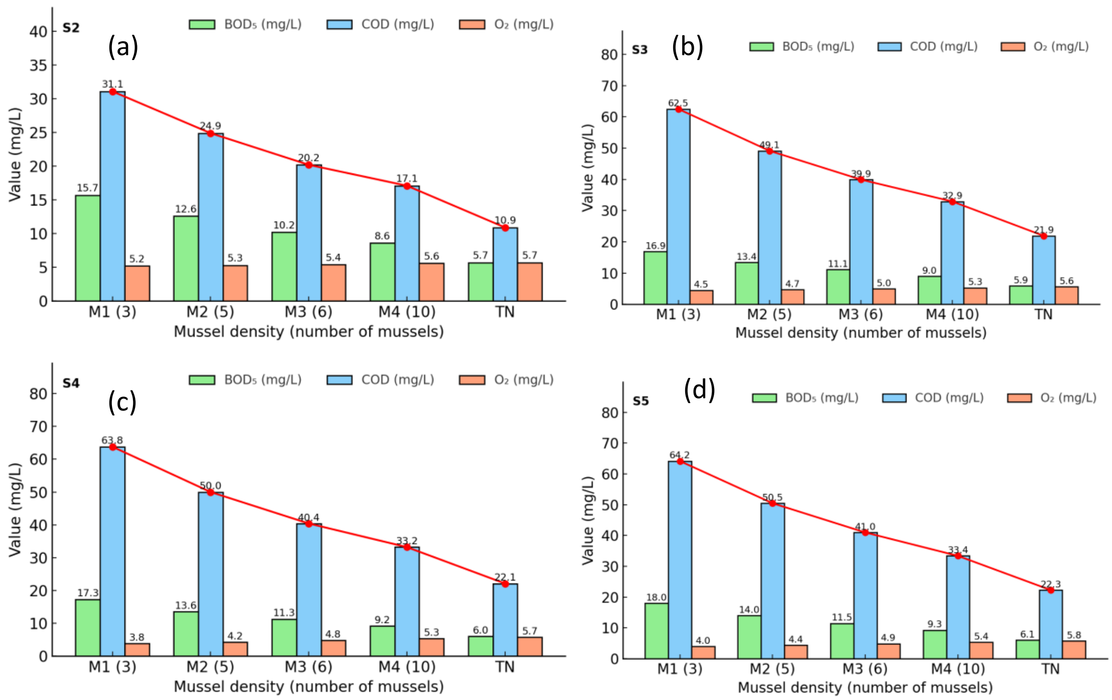


Fig. 11. Parameters of BOD_5, COD and dissolved oxygen at S2 across mussel densities (M1–M4, TN)



(a): S2 (12/02/2017), (b): S3 04/03/2017),
(c): S4(23/04/2017), (d): S5 (16/05/2017)

Fig. 12. Parameters of BOD_5, COD and dissolved oxygen at S2 across mussel densities (M1–M4, TN)

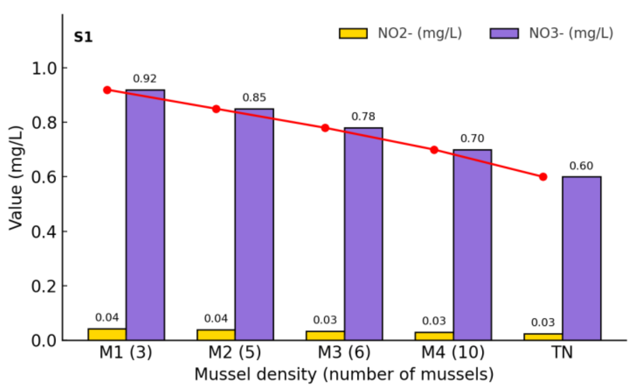
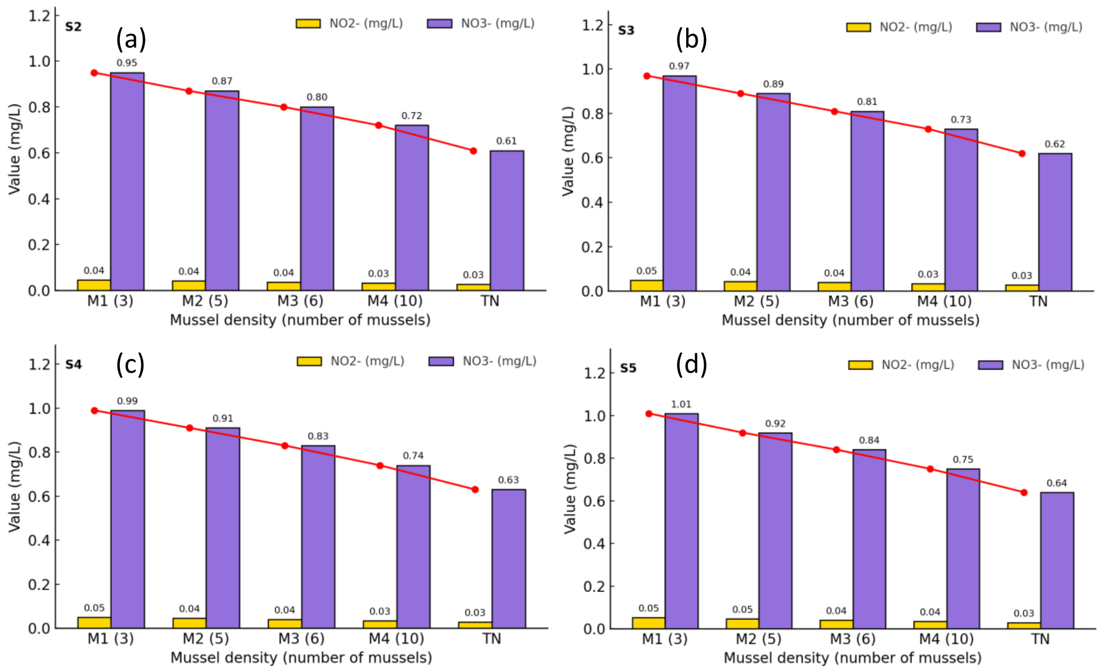


Fig. 13. Parameters of NO2- (mg/L) & NO3- (mg/L) at S1 across mussel densities (M1–M4, TN)



(a): S2 (12/02/2017), (b): S3 04/03/2017),
(c): S4(23/04/2017), (d): S5 (16/05/2017)

Fig. 14. Parameters of NO2- (mg/L) & NO3- (mg/L) at S2-S5 across mussel densities (M1–M4, TN)

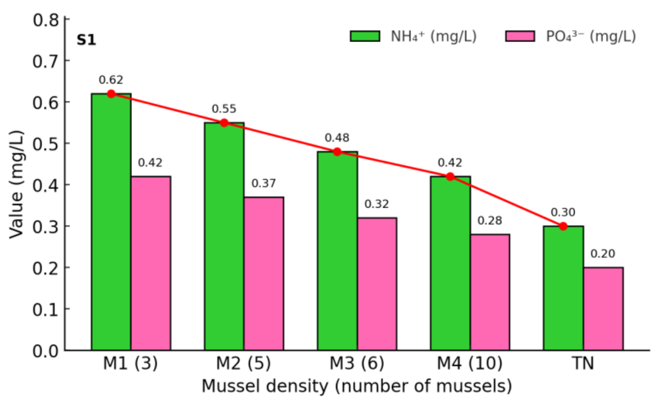
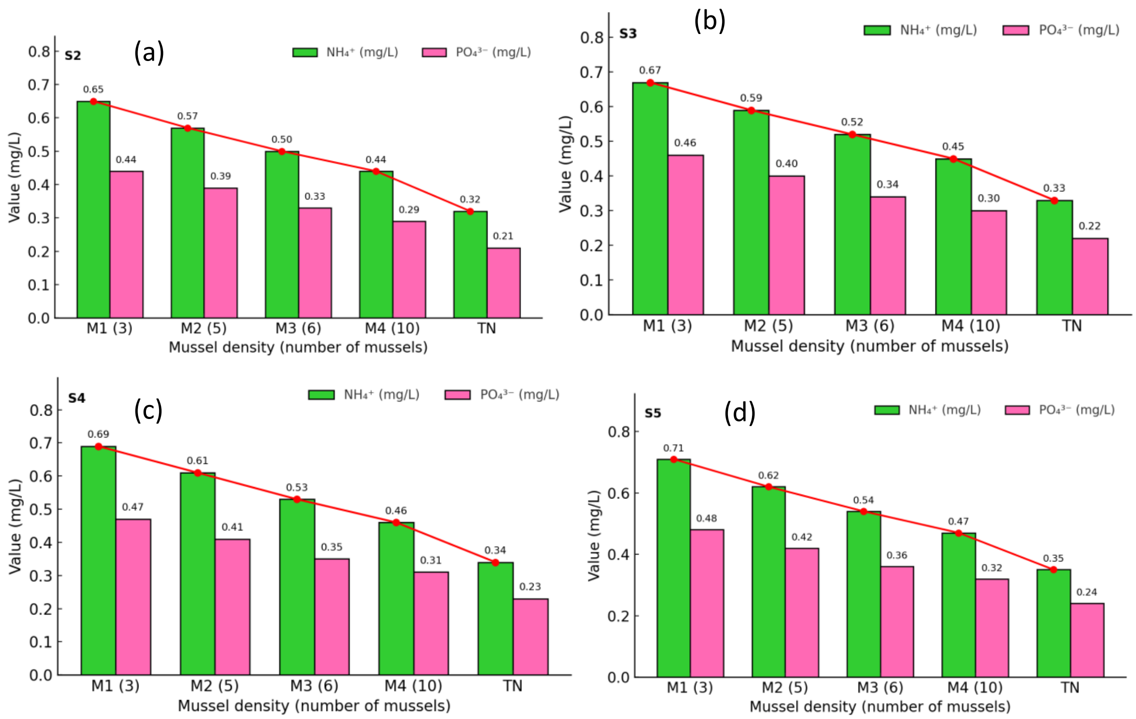


Fig. 15. Parameters of NH_4^+& PO_4^{3-} at S1 across mussel densities (M1–M4, TN)



(a): S2 (12/02/2017), (b): S3 04/03/2017),
(c): S4(23/04/2017), (d): S5 (16/05/2017)

Fig. 16. Parameters of NH_4^+& PO_4^{3-} at S2-S5 across mussel densities (M1–M4, TN)

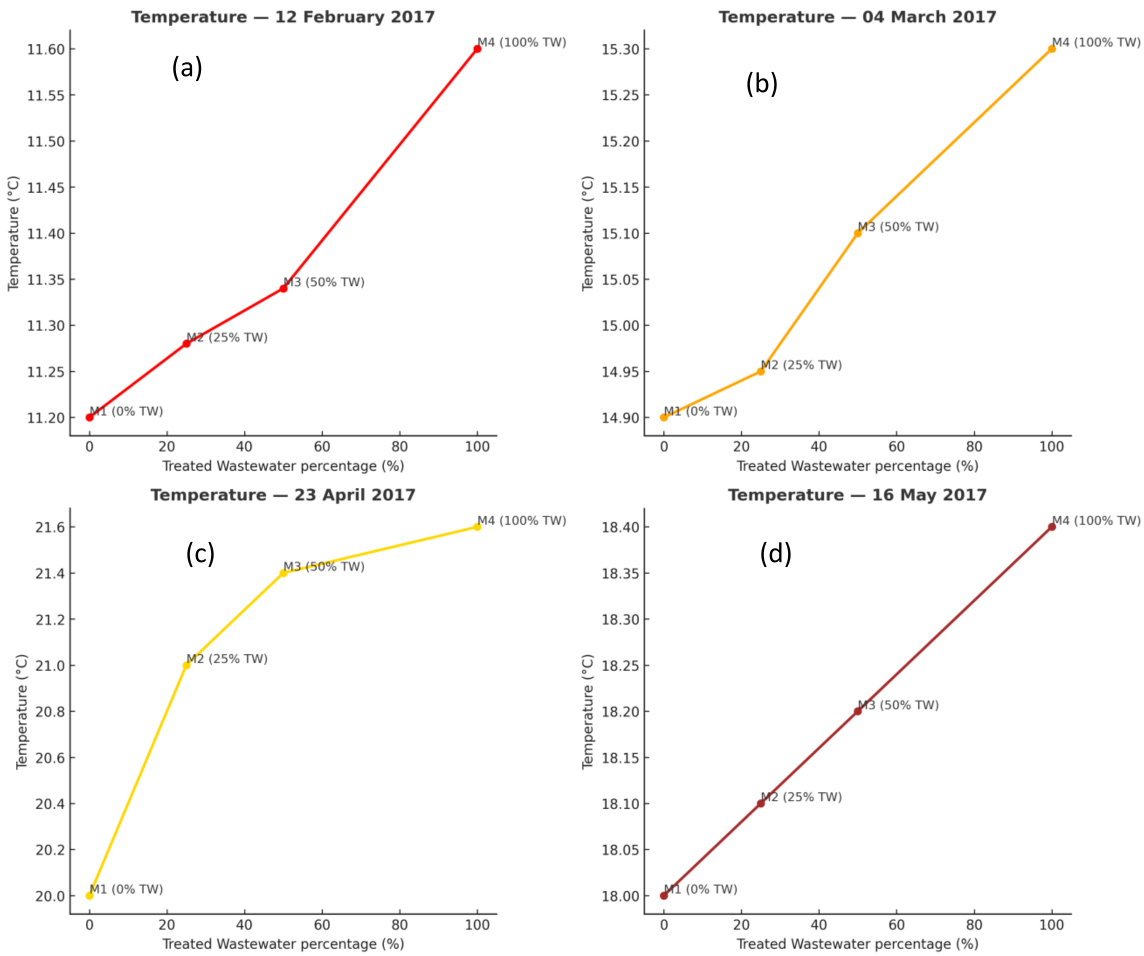
Laboratory trials demonstrated that mussel density significantly influenced water quality improvement. Increasing densities led to progressive reductions in turbidity, TSS, BOD_5, COD, and nutrients (NH_4^+, NO_2^-, NO_3^-, PO_4^{3-}) (Figs. 7-16), accompanied by higher dissolved oxygen. The strongest effects occurred in February (S2), when turbidity dropped from 44.7 NTU (control) to 15.6 NTU with 10 mussels, and TSS from 32.4 to 11.3mg/ L (Fig. 10). Similarly, BOD_5 and COD decreased most sharply in spring (S3–S4), suggesting optimal filtration under moderate temperatures. Dissolved oxygen was particularly enhanced during S4, when mussels raised concentrations from 3.8mg/ L (control) to 5.7mg/ L (Fig. 12).

Table 2. Pearson correlation matrix between physicochemical parameters of treated wastewater and filtration responses of *Anodonta cygnea*

Correlation	T°C	pH	Conduct	Turbid	Dissolved O_2	TSS	BOD_5	COD	COD/ BOD_5	NO_3-N	NO_2-N	PO_4^-	NH_4^+
T°C	**1**												
pH	-0.41	**1**											
Conduct.	0.13	0.17	**1**										
Turbid.	-0.16	0.56	0.69	**1**									
Dissolved O_2	-0.13	-0.44	**-0.91****	-0.80	**1**								
TSS	-0.23	0.63	0.71	**0.99****	**-0.83****	**1**							
BOD_5	0.30	0.25	**0.87****	0.47	-0.90	0.53	**1**						
COD	0.16	0.26	**0.98****	**0.73****	**-0.95****	**0.75****	**0.89****	**1**					
COD/BOD_5	-0.17	-0.52	-0.26	-0.28	0.49	-0.35	-0.52	-0.26	**1**				
NO_3-N	**-0.8****	**0.81****	-0.12	0.43	-0.07	0.49	-0.20	-0.07	-0.20	**1**			
NO_2-N	0.51	0.31	0.38	0.49	-0.53	0.43	0.42	0.48	-0.19	-0.15	**1**		
PO_4^-	0.25	0.49	0.55	**0.77****	-0.67	**0.72****	0.44	0.63	-0.21	0.12	**0.91****	**1**	
NH_4^+	0	0.14	**0.96****	0.60	**-0.86****	0.64	**0.86****	**0.94****	-0.24	-0.08	0.16	0.34	**1**

N. B.: r = Pearson coefficient; * $P < 0.05$; ** $P < 0.01$. Bold values indicate $|r| \geq 0.70$.
Sample size ≈ 20.

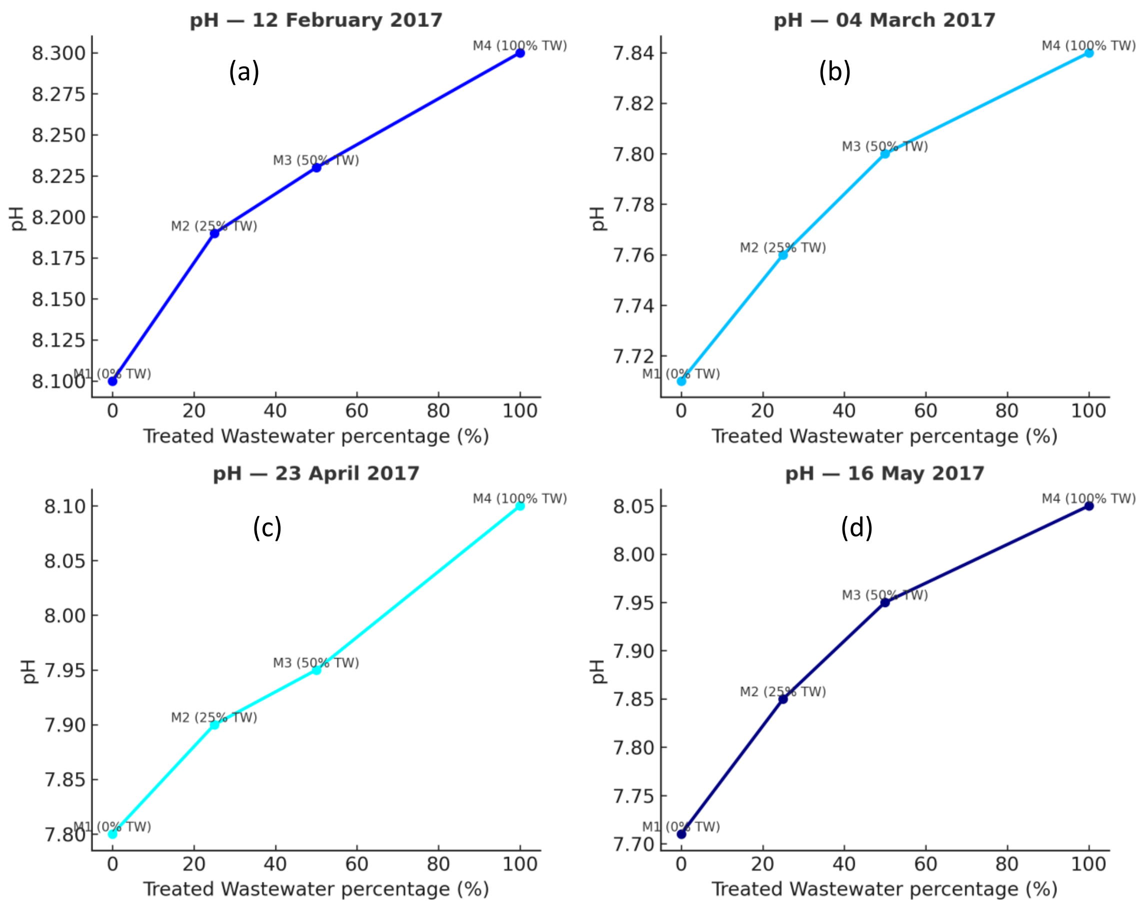
Correlation analysis (Table 2) highlighted strong relationships among key variables. Nitrite and phosphate were tightly coupled (r= 0.91, $P < 0.01$), suggesting shared sources or synchronized transformations **(Seitzinger *et al.*, 2006)**. Positive associations included conductivity with ammonium (r= 0.96) and COD with NH_4^+ (r= 0.94), confirming organic degradation as a source of reduced nitrogen **(Wetzel, 2001)**. A strong positive correlation was also observed between BOD_5 and conductivity (r= 0.87). Conversely, dissolved oxygen was negatively correlated with COD (r= –0.95), conductivity (r= –0.91), and BOD_5 (r= –0.90), reflecting the impact of organic enrichment on oxygen depletion. Strong negative associations also appeared between TSS and dissolved oxygen (r= –0.83), and between turbidity and dissolved oxygen (r= –0.80). These patterns highlight the regulatory role of *Anodonta cygnea* mussels in improving water quality by reducing suspended matter (TSS, turbidity), enhancing oxygenation, and mediating nutrient cycling **(Vaughn & Hakenkamp, 2001)**.



(a): S1 (12/02/2017), (b): S2 04/03/2017),

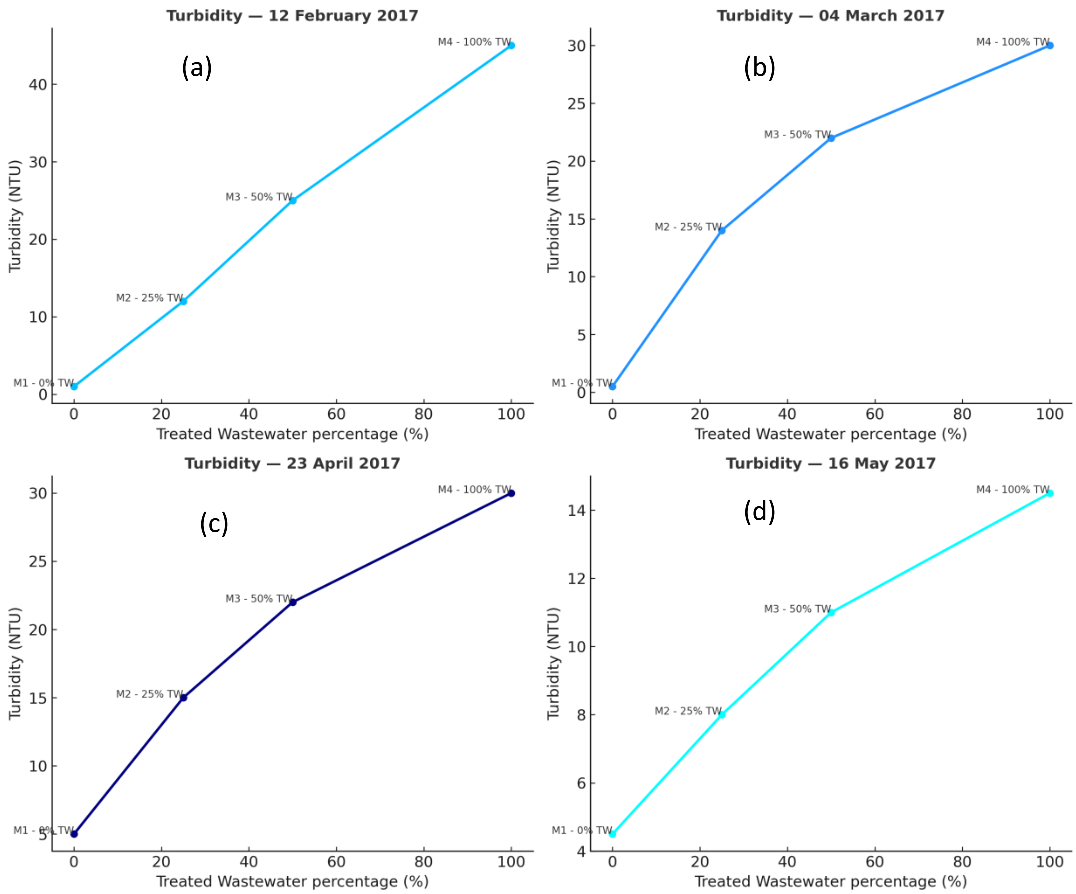
(c): S3(23/04/2017), (d): S4 (16/05/2017)

Fig. 17. Change in temperature with different treated wastewater percentages (S1–S4)



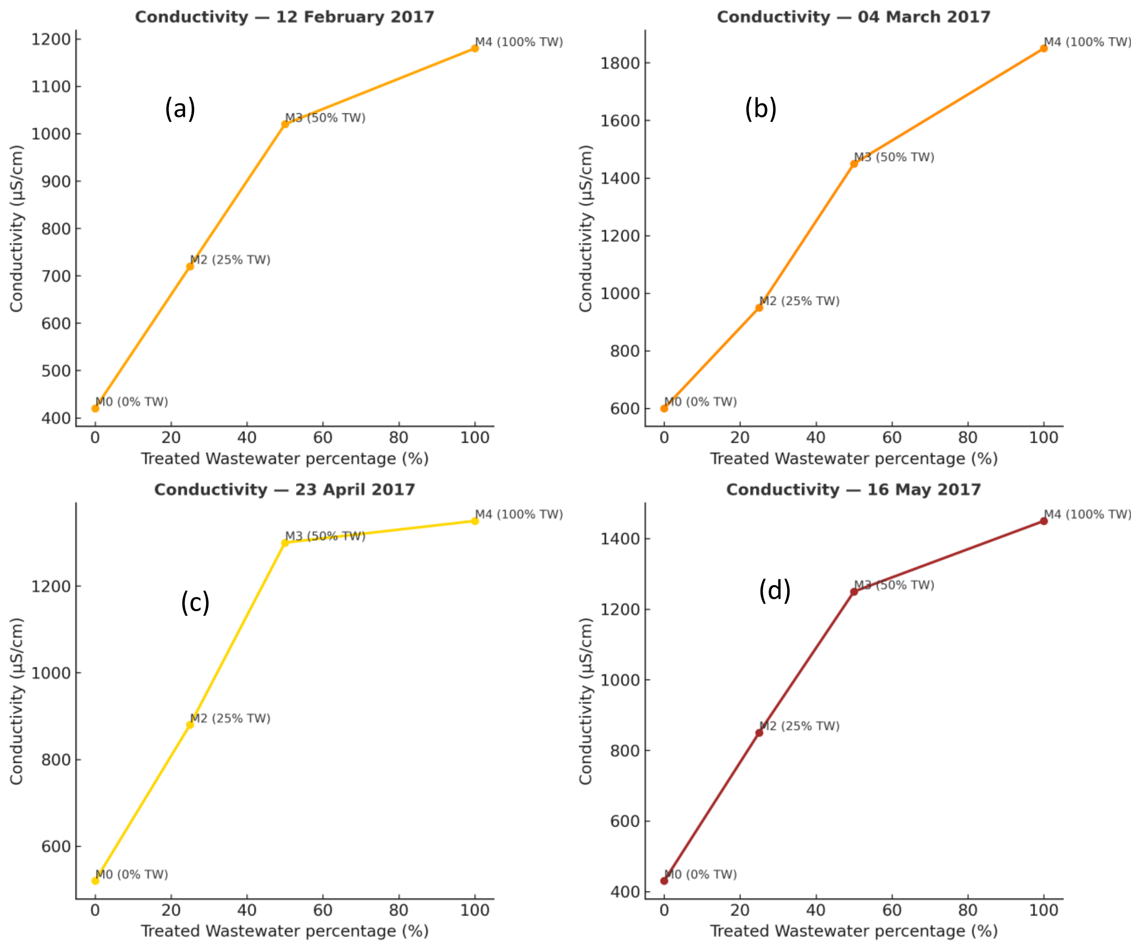
(a): S1 (12/02/2017), (b): S2 04/03/2017),
(c): S3(23/04/2017), (d): S4 (16/05/2017)

Fig. 18.Variation of pH values in response to treated wastewater proportion (S1–S4)



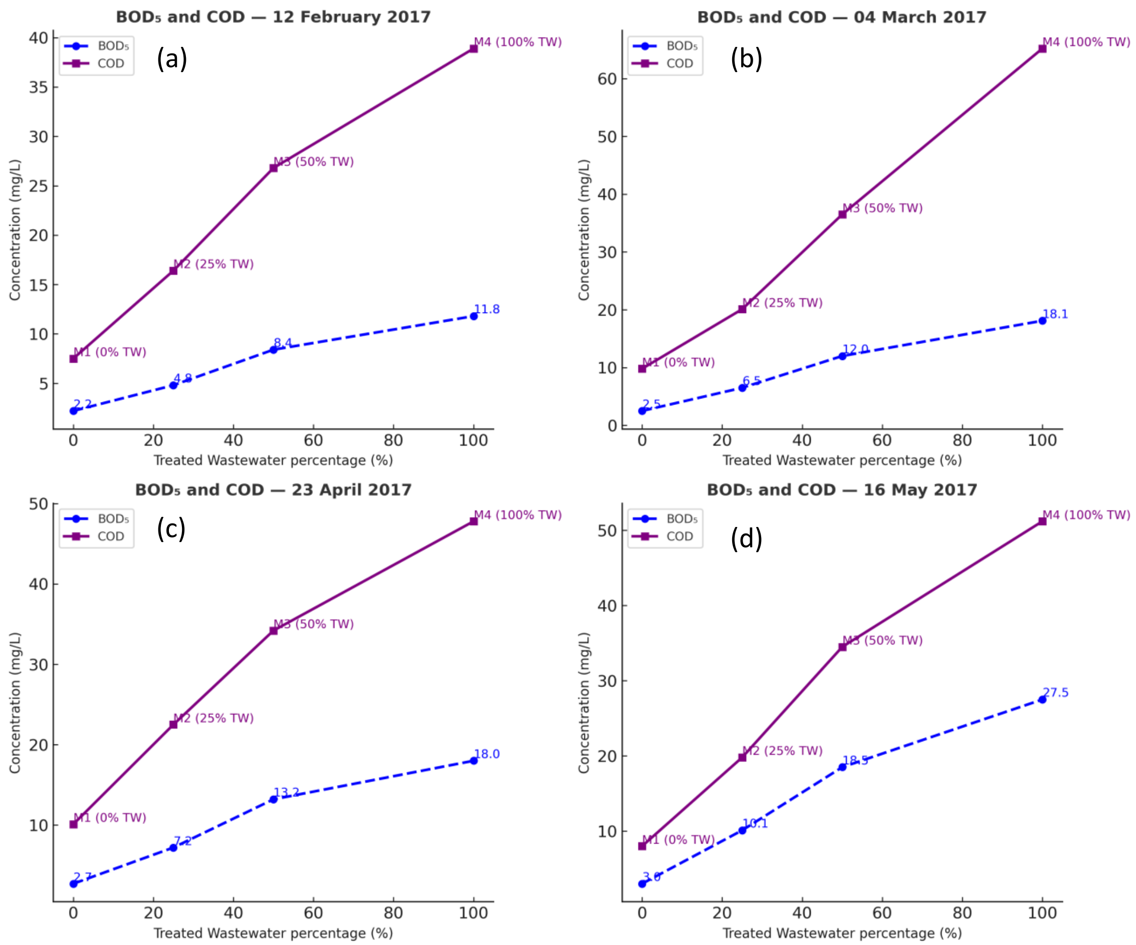
(a): S1 (12/02/2017), (b): S2 04/03/2017),
(c): S3(23/04/2017), (d): S4 (16/05/2017)

Fig. 19. Variation of turbidity with treated wastewater percentage (S1-S4)



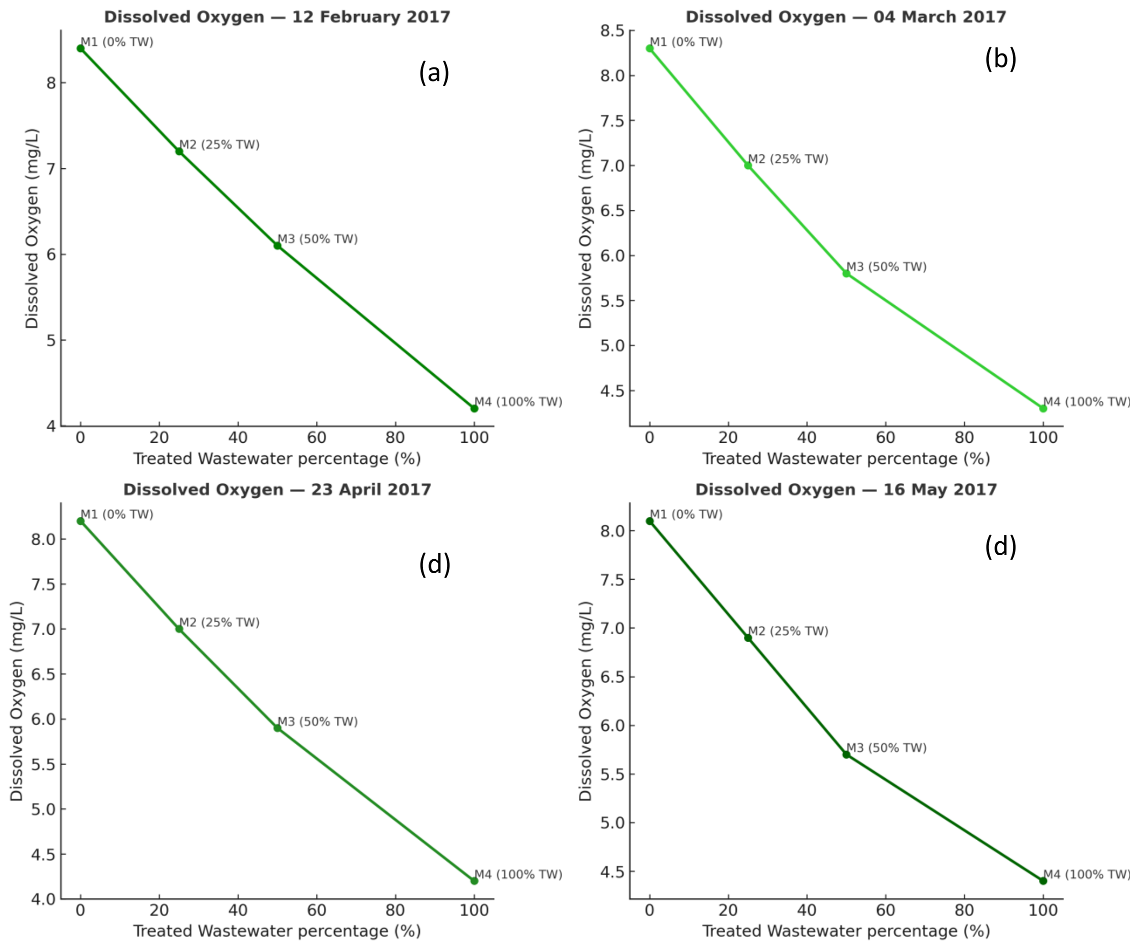
(a): S1 (12/02/2017), (b): S2 04/03/2017),
(c): S3(23/04/2017), (d): S4 (16/05/2017)

Fig. 20. Evolution of conductivity under increasing treated wastewater percentage (S1–S4)



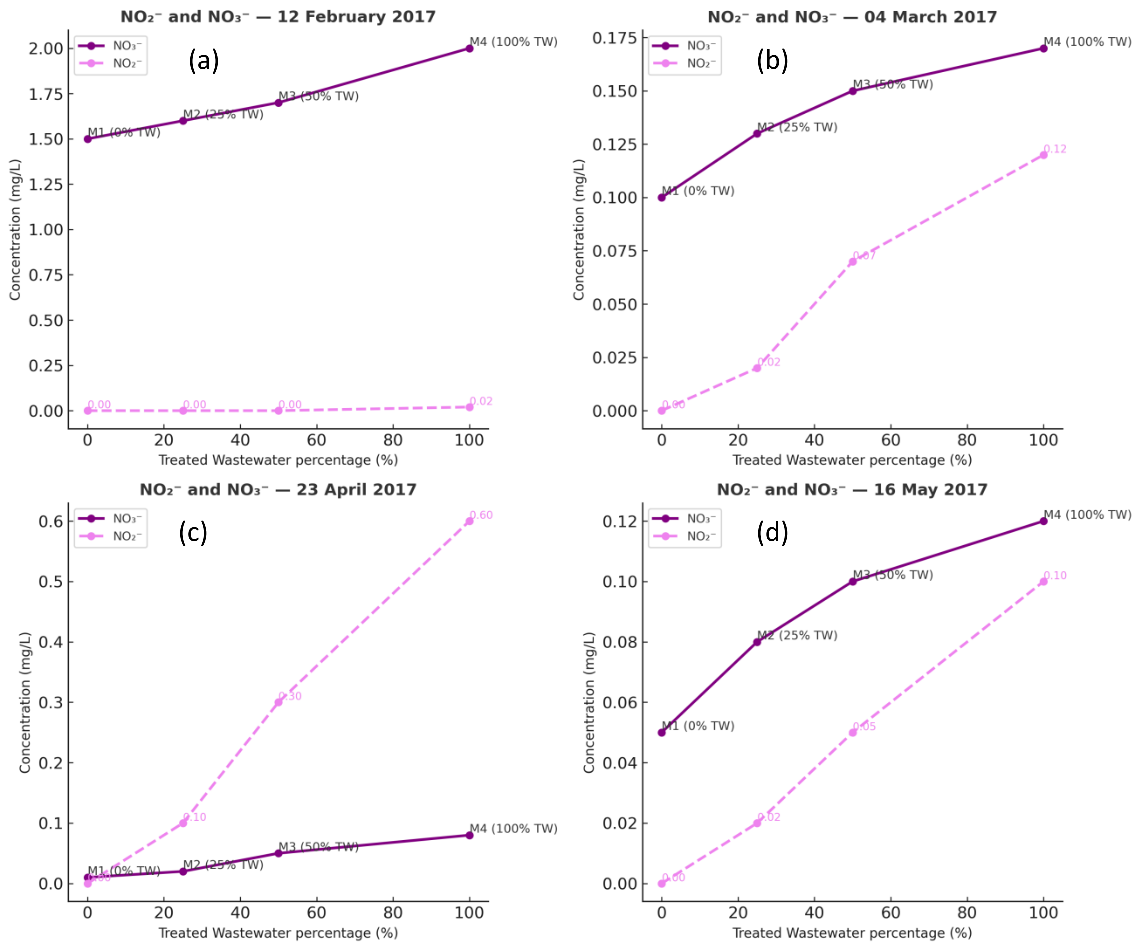
(a): S1 (12/02/2017), (b): S2 04/03/2017),
(c): S3(23/04/2017), (d): S4 (16/05/2017)

Fig. 21. Effect of treated wastewater percentage on BOD_5 and COD concentration (S1–S4)



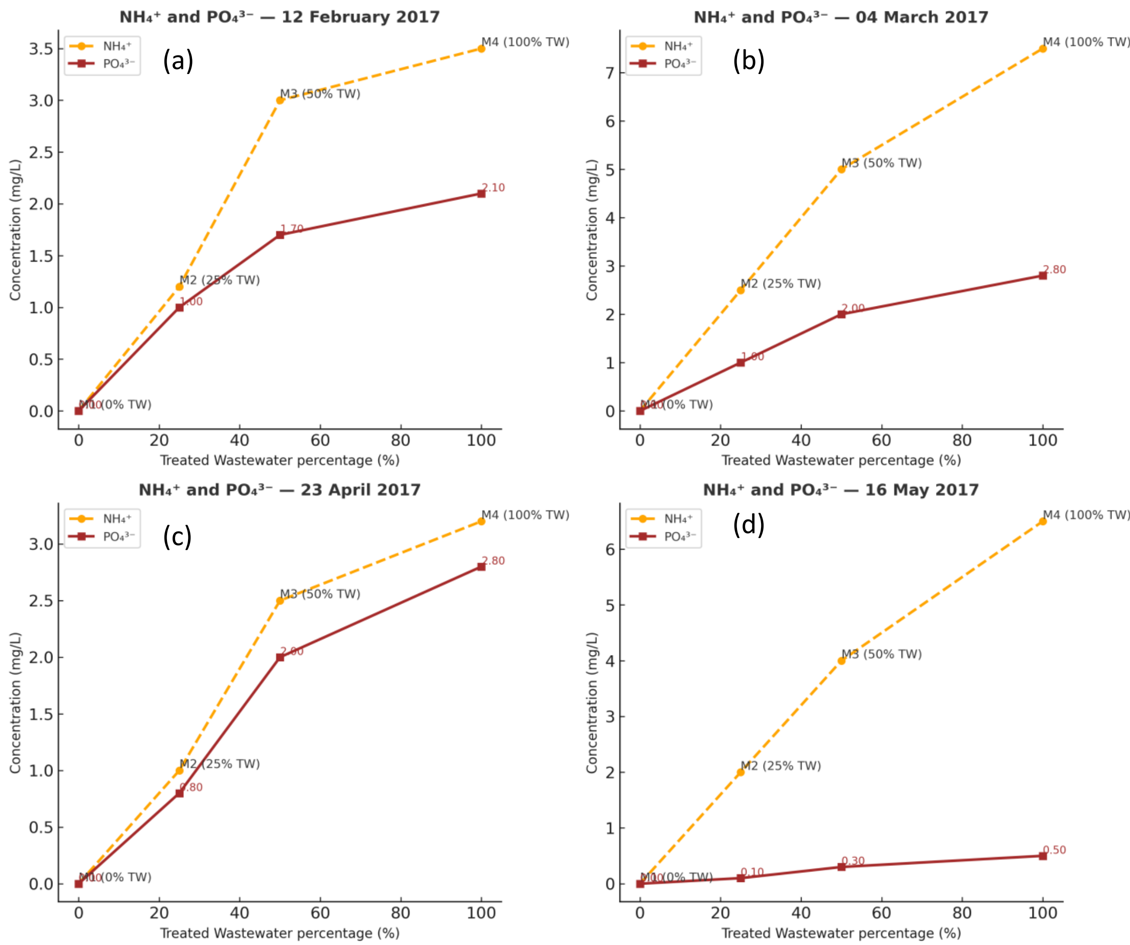
(a): S1 (12/02/2017), (b): S2 04/03/2017),
(c): S3(23/04/2017), (d): S4 (16/05/2017)

Fig. 22. Response of dissolved oxygen to treated wastewater gradient (S1–S4)



(a): S1 (12/02/2017), (b): S2 04/03/2017),
(c): S3(23/04/2017), (d): S4 (16/05/2017)

Fig. 23. Variation of nitrite and nitrate concentrations along treated wastewater percentage (S1–S4)



(a): S1 (12/02/2017), (b): S2 04/03/2017),
(c): S3(23/04/2017), (d): S4 (16/05/2017)

Fig. 24. Influence of treated wastewater percentage on phosphate levels and ammonium concentration (S1–S4)

Table 3. Pearson correlation matrix between physicochemical parameters of treated wastewater and biological responses of *Cyprinus carpio* fry

Correlation	T°C	pH	Conduct	Turbid	Dissolved O_2	TSS	BOD_5	COD	COD/ BOD_5	NO_3-N	NO_2-N	PO_4^-	NH_4^+
T°C	**1**												
pH	-0.41	**1**											
Conduct.	0.13	0.17	**1**										
Turbidity	-0.16	0.56	0.69	**1**									
DissolvedO_2	-0.13	0.44	**-0.91** **	**-0.80** **	**1**								
TSS	-0.23	0.63	**0.71** **	**0.99** **	**-0.83** **	**1**							
BOD_5	0.30	0.25	**0.87** **	0.47	**-0.90** **	0.53	**1**						
COD	0,16	0.26	**0.98** **	**0.73** **	**-0.95** **	**0.75** **	**0.89** **	**1**					
COD/BOD_5	-0.17	-0.52	-0.26	-0.28	0.49	-0.35	-0.52	-0.26	**1**				
NO_3-N	**-0.8** **	**0.81** **	-0.12	0.43	-0.07	0.49	-0.20	-0.07	-0.20	**1**			
NO_2-N	0.51	0.31	0.38	0.49	-0.53	0.43	0.42	0.48	-0.19	-0.15	**1**		
PO_4^-	0.25	0.49	0.55	**0.77** **	-0.67	**0.72** **	0.44	0.63	-0.21	0.12	**0.91** **	**1**	
NH_4^+	0	0.14	**0.96** **	0.6	**-0.86** **	0.64	**0.86** **	**0.94** **	-0.24	-0.08	0.16	0.34	**1**

N. B.: **r = Pearson coefficient; * *P*< 0.05; ** *P*< 0.01. Bold values indicate |r| ≥ 0.70. Sample size ≈ 16.**

Correlation analysis confirmed turbidity and suspended solids were almost perfectly linked (r= 0.99), indicating that particulate matter drives light attenuation **(Bilotta & Brazier, 2008)**. Conductivity correlated strongly with COD (r= 0.98) and ammonium (r = 0.96), reflecting combined mineral and organic inputs. COD and NH_4^+ were also closely related (r= 0.94), consistent with organic mineralization. Nitrite and phosphate were strongly associated (r= 0.91), suggesting nutrient remobilization. Dissolved oxygen showed negative correlations with COD (r= –0.95), conductivity (r= –0.91), BOD_5 (r= –0.90), and NH_4^+ (r= –0.86), indicating eutrophication-driven oxygen depletion **(Diaz & Rosenberg, 2008)**. These relationships reveal organic enrichment and mineral inputs as key stressors, with sublethal risks for fish performance despite the absence of mortality.

DISCUSSION

These findings confirm the role of *A. cygne* as an efficient bioremedicator, though performance is seasonally modulated **(Aldridge *et al*., 1990; Caraco *et al*., 2000)**. Exposure of *C. carpio* fry to graded concentrations of TW (25–100%) revealed strong seasonal influences. In winter (S1, ~11 °C), fish experienced minimal stress, with DO at 8.5mg/ L in controls, though turbidity (46 NTU) and ammonium (4.1mg/ L) were elevated at 100% TW. By April (S3, ~21 °C), nitrite peaked (0.67mg/ L) near chronic toxicity levels **(Camargo & Alonso, 2006)**, while phosphate rose to 3.9mg/ L, likely due to sediment release. In May (S4), relative recovery was noted with reduced turbidity (14.6 NTU), though ammonium remained high (6.9mg/ L) and DO was low (4.1mg/ L). No mortality occurred, highlighting the resilience of *C.carpio*, though sublethal effects on growth and behavior are likely to occur **(Nielsen & Thorpe, 2014)**.

Overall, the study demonstrates that both the WWTP and the additional biofiltration by *A. cygne*a substantially improved wastewater quality, reducing organic load and nutrient concentrations removal reached 60–70% under high mussel densities, though ammonium fluctuations reflected microbial regeneration processes **(Strayer, 2014)**. Seasonal variability strongly influenced treatment efficiency and organismal responses, underscoring the importance of long-term monitoring. While *C.carpio* fry exhibited remarkable tolerance to treated wastewater, sublethal impacts are likely under chronic exposure. The integration of bivalve biofiltration into conventional treatment systems provides a promising strategy for sustainable aquaculture and wastewater reuse in semi-arid environments.

CONCLUSION

This study provided an integrated assessment of the Sidi Bel Abbès activated sludge wastewater treatment plant (WWTP), complemented by the biofiltration capacity of *Anodonta cygnea* and the physiological tolerance of *Cyprinus carpio* fry to treated effluents. Conventional treatment achieved substantial reductions in turbidity, total suspended solids (TSS), biochemical oxygen demand (BOD_5), and chemical oxygen demand (COD), with removal efficiencies generally exceeding 90%. These results confirm the plant's ability to produce effluent relatively stable in terms of pH and temperature, suitable for controlled aquaculture applications. Nonetheless, residual ammonium, nitrite, and phosphate concentrations remained elevated under certain conditions, reflecting incomplete nitrification and highlighting potential eutrophication risks. The inclusion of *A. cygnea* significantly enhanced water quality by reducing particulate matter and nitrogenous compounds in proportion to mussel density, thereby reinforcing its role as an ecological engineer in water purification. From a biological perspective, *C. carpio* fry maintained high survival and resilience across all exposure levels, even under suboptimal conditions, indicating their potential for use in aquaculture systems based on treated wastewater. However, continuous monitoring of key parameters is necessary to avoid sublethal stress and to ensure long-term ecosystem stability. Overall, combining conventional wastewater treatment with mussel biofiltration offers a promising, sustainable approach for circular water reuse in aquaculture. Optimizing unit design, regulating mussel density, and implementing rigorous monitoring could enhance system efficiency and contribute to water resource security and environmental protection in semi-arid regions.

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