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Biotreatment of Sewage using the Amazon Sailfin Catfish, *Pterygoplichthys pardalis*

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Abstract: Purpose: The present study examines the potential uses of Amazon Sailfin Catfish *Pterygoplichthys pardalis* in wastewater treatment. The main objective is to investigate the efficiency of *P. pardalis* in the biotreatment of sewage. Methods: The treatment of sewage with *P. pardalis* was carried out by analyzing the parameters such as total solids, total suspended solids, total dissolved solids, pH, BOD₃ 30° C and phosphates. The biotreatment efficiency of the fish *P. pardalis* was studied using different concentrations of sewage (20, 40, 60 and 80%) after 2, 4, 6 and 8 days of treatment. Results: The investigation revealed that after treatment with *P. pardalis*, the treated sewage had lower values of all the parameters than that of untreated sewage.

Conclusion: It can be concluded that *P. pardalis* is efficient in the remediation of sewage.

Keywords: *Pterygoplichthys pardalis*; Biotreatment; Sewage; Physico-chemical parameters.

1. Introduction

Water is essential for all living organisms but still water pollution and the destruction of aquatic ecosystems continue to increase. Deterioration of water quality is a major problem in the global context due to industrialization, globalization, population growth, urbanization and warfare combined with increased wealth and extravagant lifestyles [1]. In the next twenty five years the world human population is expected to grow by almost two billion, a growth which can be seen mainly in urban centres in developing countries [2]. As a result of this unprecedented growth, fresh water will become increasingly scarce and a primary constraint for enhanced food production [3]. The use of treated wastewater for crop irrigation has been suggested as one of the possible ways out of a looming water crisis WHO [4]. The construction, operation and maintenance costs of wastewater treatment plants are high and in many developing countries wastewater is used without any form of treatment [5].

The discharge of effluents from wastewater treatment plants (WWTPs) has major detrimental effects on the health of aquatic ecosystems. WWTP outfall can deposit large amounts of organic matter and nutrients in receiving water systems. Increased nutrient loading may lead to eutrophication [6] and temporary oxygen deficits [7]. Increased organic matter can alter energy relationships in aquatic systems disrupting community structure and function [8]. Effluent discharge can also deposit sand and grit in water systems, affecting physical characteristics of sediment. The discharge itself can alter natural flow regimen, particularly when it enters waterways during periods of low natural flow. In this regard, Brooks, *et al.* [9] highlighted biomonitoring challenges of effluent-receiving streams with ephemeral or seasonally variable flows. Assessing the effects of effluent discharge on the health of receiving aquatic systems is of considerable environmental consequence [10, 11].

Wastewater contains significant amounts of ammonium, of which only a small proportion is oxidized by conventional treatment plants [12]. Ammonium oxidation and the decomposition of organic compounds within receiving waters can have a significant draw-down effect on dissolved oxygen, with potentially detrimental effects on aquatic biota. Domestic sewage is made up of organic carbon, either in solution or as particulate matter. Particles of 1 to 100 nm remain in colloidal suspension and during treatment become adsorbed on to the flocs of the activated sludge. The bulk of the organic matter is easily biodegradable, having proteins, amino acids, peptides, carbohydrates, fats and fatty acids. The average carbon to nitrogen to phosphorus ratio(C: N: P ratio) is stated as approximately as 100: 17: 5 or 100: 19: 6. This is also ideal for the growth of activated sludge bacteria. However, industrial wastewaters are more variable in composition. Those produced by brewing and pulp and paper industries are deficient in nitrogen and phosphates. These nutrients need to be added to achieve the correct ratio for microbial growth and optimal treatment [13].

There is deficit of water throughout the world and hence there is a need for recycling of waste water whenever possible [14]. Several fresh water fish species have been cultivated in aquaculture ponds receiving human waste,

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including common carp (*Cyprinus carpio*), Indian major carps (*Catla catla*, *Cirrhinus mrigala* and *Labeo rohita*), Chinese silver carp (*Hypophthalmichthys molitrix*), Bighead carp (*Aristichthys nobilis*), Grass carp (*Ctenopharyngodon idella*), Crucian carp (*Carassius auratus*), Nile carp (*Osteochilus hasseltii*), Tilapia (*Oreochromis spp.*), Milkfish (*Chanos chanos*), Catfish (*Pangasius spp.*), Kissing gouramy (*Helostoma temmincki*), Giant gourami (*Osphronemus goramy*), Silver barb (*Puntius gonionotus*) and the freshwater prawn (*Macrobrachium lanchesterii*) [15]. *Azolla* has been proved as an efficient agent in the biofiltration of various toxic metals [16]. *Vulgaris* was tested in the removal of nitrogen and phosphorus compounds as well as heavy metals from waste water. Recycled waste water can be used in aquaculture [17]. *Chlorella vulgaris* is a green freshwater unicellular microalga known for its fastest growth. Many authors have reported that *C.vulgaris* can be used in removal of nitrogen and phosphorus compounds as well as heavy metals from wastewater [18]. Aquatic macrophytes play an important role in structural and functional aspects of aquatic ecosystems by many ways. The ability to take up heavy metals makes them interesting research candidates especially in treatment of industrial effluents and sewage waters [19].

Pterygoplichthys spp. can be found in a wide variety of habitats, ranging from relatively cool, fast-flowing and oxygen-rich highland streams to slow-flowing, warm lowland rivers and stagnant pools poor in oxygen. They are tropical fish and their populations are typically limited by their lower lethal temperature which has been found to be 8.8-11°C in some species [20]. They can thrive in a range of acidic to alkaline waters (pH 5.5 to 8.0) [21]. They are often observed in soft waters, but can adapt very quickly to hard waters. *Pterygoplichthys* spp. are also highly tolerant to poor water quality and are commonly found in polluted waters [22]. They are known to use outflow from sewage treatment plants as thermal refugia and can readily adapt to changing water quality [23]. *Pterygoplichthys* spp. may be found in lowlands to elevations of up to 3,000 m [24] and some species are salt tolerant [21]. Hence in the present study, the possibilities of employing the tank cleaner, *P. pardalis* as an agent of biotreatment in the remediation of sewage were explored by analyzing changes in certain chemical parameters.

2. Materials and Methods

2.1. Collection of Sample

Sewage samples were collected from nearby drainage in sterile containers and immediately brought to the laboratory for analysis.

Sample analysis

Sewage and ground water used for dilution were analyzed for physical and chemical parameters like colour, odour, pH, temperature, BOD₃ 30°C, total solids, total dissolved solids, total suspended solids and phosphates [25].

Colour

Colour in water resulted from the presence of metallic ions (iron and manganese), humus, peat materials, plankton, weeds and industrial wastes. True colour is the colour of water from which turbidity has been removed. Apparent colour may be due to substances and suspended matter. Untreated sample was taken in a 50ml Nessler tube and matched with standard colours.

Odour

It is the stimulation of human receptor cells by the stimulating sample. Different types of odours are H₂S smell, rotten egg smell, algae odour, oil smell etc. The sewage sample was tested for the type of odour.

pH

The pH of sewage was determined using a pH meter calibrated with known buffer solutions.

Temperature

Temperature of the sample was determined using thermometer.

Total Solids (TS)

The initial weight (W_1) of the porcelain or evaporating dish was taken and 100ml of water sample (V_1) was taken. It was evaporated in a water bath and kept at 103 - 105°C for one hour in an oven. Then it was kept in a desiccators cooled and the final weight (W_2) was taken.

$$\text{Formula} = \text{TS (mg/l)} = \frac{W_2 - W_1}{V_1} \times 1000$$

Total Dissolved Solids (TDS)

The initial weight (W_1) of the porcelain or evaporating dish was taken and 100ml of filtered (Whatman No.1) water sample (V_1) was taken. It was evaporated in a water bath and kept at $180 \pm 2^\circ\text{C}$ for one hour in an oven. Then it was kept in a desiccators, cooled and the final weight (W_2) was taken. Using the following formula TDS level was calculated.

$$\text{Total dissolved solids (mg/l)} = \frac{W_2 - W_1}{V_1} \times 1000$$

Total Suspended Solids (TSS)

$$\text{Total Suspended Solids (mg/l)} = (\text{TS} - \text{TDS})$$

BOD₃ 30°C

Required volume of distilled water was taken in a suitable bottle and 1 ml each of phosphate buffer, magnesium sulphate, calcium chloride and ferric chloride solution per liter of water were added.

Dilution were prepared either in graduated cylinder or in volumetric flask and mixed well with plunger type mixing rod. Fill two sets of BOD bottles with this water and add one ml of allylthiourea solution to each bottle. Determine the dissolved oxygen content in one set immediately the Winkler's method of oxygen estimation.

Incubate the other set of BOD bottles at 20°C for five days in a BOD incubator. Take out the bottles after 5 days and determine immediately their dissolved oxygen content.

$$\text{BOD}_3 \text{ } 30^\circ\text{C (mg/l)} = (\text{Initial DO} - \text{Final DO}) \times \text{Dilution factor}$$

Phosphates

25 ml of sample was taken in an Erlenmeyer flask and evaporated to dryness. It was cooled and dissolved in 1 ml of perchloric acid. The flask was gently heated so that the contents become colourless. After cooling 10 ml of distilled water and 2 drops phenolphthalein indicator were added. It was titrated against sodium hydroxide solution until the appearance of slight pink colour. It was made up to 25 ml by adding distilled water. Later 1 ml of ammonium molybdate solution and 3 drops of stannous chloride solution were added. After the appearance of blue colour and the absorbance was read on a spectrophotometer at 690 nm. Simultaneously distilled water blank was run in similar manner.

The Amazon Sailfin Catfish (*Pterygoplichthys pardalis*) was the agent for biotreatment. *Pterygoplichthys pardalis* is a facultative air breather. The fish of 13.5 to 15.2 cm length with a weight of 8 to 13.09g were used for experiments. The fish were collected from Kadachanendal, Madurai. 20, 40, 60 and 80% concentrations were prepared by using ground water for dilution. The chemical parameters were analyzed after 2, 4, 6 and 8 days of treatment of sewage.

2.2. Statistical Analysis

Two way Analysis of variance (ANOVA) for the parameters Total dissolved solids, Total suspended solids Total solids, BOD and phosphates was performed using MS Excel. Variability was considered significant only when the statistic value was greater than the tabulated value at P is less than or equal to 0.05.

3. Results and Discussion

Table 1 divulges the physical and chemical characteristics of sewage and dilution water. Sewage sample was having higher levels of total solids, total dissolved solids, total suspended solids, BOD₃ 30⁰C and phosphates than that of dilution water. The sewage was block in colour with objectionable odour. The temperature of sewage was more than that of dilution water

Initial pH was found to be 7 and it increased upto 7.4 at the end of eight days of treatment (Fig.1). Increase in pH was observed in the control after 4, 6 and 8 days of treatment whereas the pH remained the same in the experimental set with the increase in treatment period.

Lowest level of total solids of 60mg/L at 20% concentration and the highest value of 150mg/L at 60% concentration of sewage were noted after eight days of treatment (Fig.2). Decline of total solids in both control and experimental sets was noticed. Levels of total dissolved solids decreased from 74 to 10 mg/L during treatment of sewage with *P. pardalis*. 10mg/L of total dissolved solids was recorded in 20% concentration of sewage after eight days of treatment. Decline in the level of total dissolved solids was observed in both control and experimental sets with the increase in treatment period (Fig.3).

The level of total suspended solids has been gradually reduced for all the concentrations of sewage. Lowest level of total suspended solids of 40mg/L was observed in 80% concentration of sewage after eight days of treatment (Fig.4). The fish was efficient in reducing the level of total suspended solids in 80% sewage after eight days of treatment.

Levels of BOD₃ 30⁰C have been reduced gradually during treatment period. But the decline in the level of BOD was noticed in both control and experimental sets and it was gradual (Fig.5). The level of phosphates was highly fluctuating. Maximum level was observed on sixth day while reduction was noticed after eight days of treatment. Lowest level of phosphates of 110mg/L was found at 20 and 40% concentrations of sewage after eight days of treatment (Fig.6). There were fluctuations in the level of phosphates in the control set while the decline in the experimental sets was concrete after six and eight days of treatment.

Wastewater is a combination of liquid or water-carried wastes originating in the sanitary conveniences of houses, commercial or industrial facilities and institutions. Untreated wastewater generally contains high levels of organic compounds, numerous pathogens, nutrients and toxic compounds. It thus poses environmental and health hazards and has to be treated properly before final disposal. The ultimate aim of wastewater management is the protection of environment in a manner agreeable with public health and socio-economic concerns. Wastewater treatment is becoming more critical due to diminishing water resources, increasing wastewater disposal costs and stricter discharge regulations that have lowered permissible contaminant levels in waste streams. The municipal sector consumes significant volumes of water, and generates large amounts of wastewater [26]. The analysis of physico-chemical characters could help in understanding the quality of water bodies [27].

pH is one of the most important operational water quality parameters. If pH is above 7, this will indicate that the water is probably hard containing calcium and magnesium [28]. In the present work the highest pH value of 7.6 was recorded in sewage. Temperature in the water is important because of its effects on the chemistry and biochemical reactions in organisms 24⁰C was found in sewage samples. Water and waste waters are subjected to the effect of ambient temperature and can be warm during summer. The temperature of water affects the efficiency of treatment. In cold temperature, the viscosity increases, which in turn, diminishes the efficiency of settling of the solids that the

water contains because of the resistance that the high viscosity offers to the downward motion of the particles as they settle [29].

Dissolved oxygen is one of the important and critical characteristics of water quality assessment. Its presence is essential to maintain the higher forms of biological life and to keep proper balance of various populations, thus making the aquatic system healthy [30]. The entry of dissolved oxygen into water body is mainly through direct diffusion from the air and photosynthetic evaluation by aquatic autotrophs. Increased organic matter results in excess oxidation of organic matter to carbon dioxide and water which creates an atmosphere of oxygen depletion and high BOD levels. The highest value of BOD₃ 30⁰C was recorded in sewage during initial day after treatment with *P. pardalis* and the level of BOD₃ 30⁰C was reduced later. Higher values can be attributed to increased discharge of wastes into drains. Similarly, higher contents of organic load as well as high proliferation of microorganisms are the causative factors for maximum BOD levels [31].

Highest value of total solids was recorded in sewage during initial day after treatment with *P. pardalis* and later the levels were reduced. Total solids in waste water represent the colloidal form and dissolved species. The probable reason for the fluctuation of total solids and subsequently the dissolved solids may be the content collision of these colloidal particles [32]. Total suspended solids do not represent floating matters that remain on top of water layer. They are under suspension and remain in water column and play an important role in water and waste water treatment. Their presence in water causes depletion of oxygen levels [32]. Highest value of total suspended solids was recorded in sewage during initial day while they were reduced after treatment with *P. pardalis*. Level of total dissolved solids in sewage has been decreased gradually from 74 to 10 mg/l during treatment with *P. pardalis*. High level of TDS may impart taste or odour. Dissolved solids in wastewater destined for agriculture purposes might be as high as 2000 mg/ L but not more than this limit [33].

Phosphates come from fertilizers, pesticides, industry and cleaning compounds. Natural sources include phosphate containing rocks and solid or liquid wastes. These are classified as orthophosphates, condensed phosphates and originally bound phosphates. The highest level of phosphates was recorded in sewage during initial day and after treatment with *pardalis* the level of phosphates was reduced., Murdoch, *et al.* [34] reported high levels of both phosphates and nitrates leading to eutrophication, which increased algal growth and reduced dissolved oxygen in water.

Table 2 exhibits two way Analysis of variance (ANOVA) for the factors total dissolved solids, total suspended solids, total solids, BOD and phosphate with treatment period and concentration of sewage as variables. The variations due to treatment period and concentration were found to be statistically significant for all the factors.

Several species of fish were tested in waste water treatment. The selection reflects local culture rather than fish optimally-suited to such environments. Chinese carps and Indian major carps are the major species in excreta-fed systems in China and India, respectively. In some countries, polyculture of several fish species is practiced. Tilapia is generally cultured to a lesser extent than carps in excreta-fed systems although, technically, they are more suitable for this environment as they can tolerate adverse environmental conditions than carp species. Milkfish have been found to have poorer growth and survival compared to Indian major carps and Chinese carps in ponds fed with stabilization pond effluent in India [15].

Edwards [15] reported a thorough review on various fish species which can be cultured in ponds fed with human waste. Considerable confusion still exists with regard to fish feeding on natural food. Although fish are generally divided into types according to their natural nutritional habits as those that feed on phytoplankton, or zooplankton or benthic animals and several species are known to feed on whatever particles are suspended in water. There is also uncertainty about the types of phytoplankton eaten by filter-feeding fish. Although blue-green algae are thought to be indigestible to fish, Tilapia has been shown to readily digest these algae and there is evidence that silver carp can do the same [15].

Fish mortality in a waste-fed pond can result from three possible causes. First, the depletion of oxygen can be caused by an increase in organic load. Second, the depletion of oxygen overnight may be due to the respiratory demand of phytoplankton grown in response to an increase in inorganic nutrients. The third cause is high ammonia concentration in waste feed. All these three causes of fish mortality have been reported in sewage-fertilized fish ponds. The sensitivity of fish to low levels of dissolved oxygen (DO) varies with species, life stages (eggs, larvae, adults) and life processes (feeding, growth, and reproduction). A minimum constant DO level of 5 mg/l is considered satisfactory, although an absolute minimum consistent with the presence of fish is probably less than 1 mg/l [35]. Fish cultured in waste-fed ponds were able to tolerate very low levels of DO for at least short periods of time, with air-breathing fish such as walking catfish *Clarias batrachus* being the most tolerant, followed by tilapia, carps, channel catfish and trout. Reducing phytoplankton biomass to maintain a reasonable level of DO in the early morning hours might well depress fish growth more than exposure to a few hours of low DO.

Unionized ammonia is toxic to fish in the range of 0.2 to 2.0 mg/l [35]. However, the tolerance of different species of fish varies, with tilapia species being least affected by high ammonia levels. Bartone, *et al.* [36] found that satisfactory growth and survival of tilapia was possible in fish ponds fed with tertiary effluent in Lima, Peru when the average total ammonia concentration was less than 2 mg N/l (Nitrogen per liter) and the average unionized ammonia concentration was less than 0.5 mg N/l, with the latter only exceeding 2 mg N/l for short periods. In ponds receiving large quantities of organic matter, sediments tend to accumulate and release anaerobic breakdown products, such as methane and sulphides, which can inhibit fish growth. Bottom feeding fish, such as common carp are most affected if the macrozoobenthos disappear.

It is generally believed that holding fish in clean water ponds for several weeks at the end of the growing cycle will remove residual objectionable odours and pathogens and provide fish acceptable for market. However, there is a lack of data on depuration practice and experimental assessment. Evidences suggest that depuration of heavily contaminated fish with bacteria in muscle tissue will not be effective [15]. Relatively short depuration periods of one to two weeks do not appear to remove bacteria from the fish digestive tract. Considering the lack of verification of the effectiveness of depuration as a health protective measure, Edwards [15] had not included it in his suggested strategies for safeguarding public health in aquaculture. The armoured sucker catfish *P. pardalis* has firmly established itself in polluted urban lakes and rivers growing to a large size. It is usually sold as a cheap catfish to clean algae from community tanks. They are long lived and peaceful and so safe with most other tank inhabitants. They do not eat plants although they may uproot them occasionally while moving about [37].

4. Conclusion

The tank cleaner fish, *P. pardalis* in 20,40,60 and 80 % concentration of sewage after 2,4,6 and 8 days of treatment was able to cause changes in the pH, total solids, total dissolved solids, total suspended solids, BOD₃ 30°C and phosphates. Both sewage concentration and treatment period were able to result in statistically significant variation in all these parameters. Hence this fish can be successfully used to extract nutrients from sewage and employed in the remediation of sewage. Bioremediation by *P.pardalis* may provide an economical and environmentally sustainable treatment method in future.

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Table-1. Physico – chemical characteristics of sewage and ground water used for dilution

S.No	Parameters	Values	
		Sewage	Dilution Water
1.	Colour	Black	Colourless
2.	Odour	Objectionable	-
3.	pH	7.6	7
3.	Temperature (°C)	24	23
4.	Total solids(mg/L)	617	70
6.	Total dissolved soilds (mg/L)	154	44
7.	Total suspended solids (mg/L)	463	26
8.	BOD ₃₀ ⁰ C (mg/L)	21.4	8.025
9.	Phosphates (mg/L)	900	490

Table-2. Two way analysis of variance for the factors with the variables, treatment period and sewage concentration for *P. pardalis*.

Factor	Source of variation	Df	MS	Calculated F value	Table F value	Level of Significance
Total Solids	Treatment period	3	11550	13.16	3.862548	Significant
	Concentration	3	10797	12.30	3.862548	Significant
Total Dissolved Solids	Treatment period	3	810.08	51.25	3.862548	Significant
	Concentration	3	1514.4	95.81	3.862548	Significant
Total Suspended Solids	Treatment period	3	9739.7	4.70	3.862548	Significant
	Concentration	3	7216.8	3.48	3.862548	Significant
BOD ₃ 30°C	Treatment period	3	188.61	315.7	3.862548	Significant
	Concentration	3	173.16	289.9	3.862548	Significant
Phosphates	Treatment period	3	383383	3.184	3.862548	Significant
	Concentration	3	12250	0.101	3.862548	Significant

Fig-1. Changes in the levels of pH in sewage after treatment with *P. pardalis*

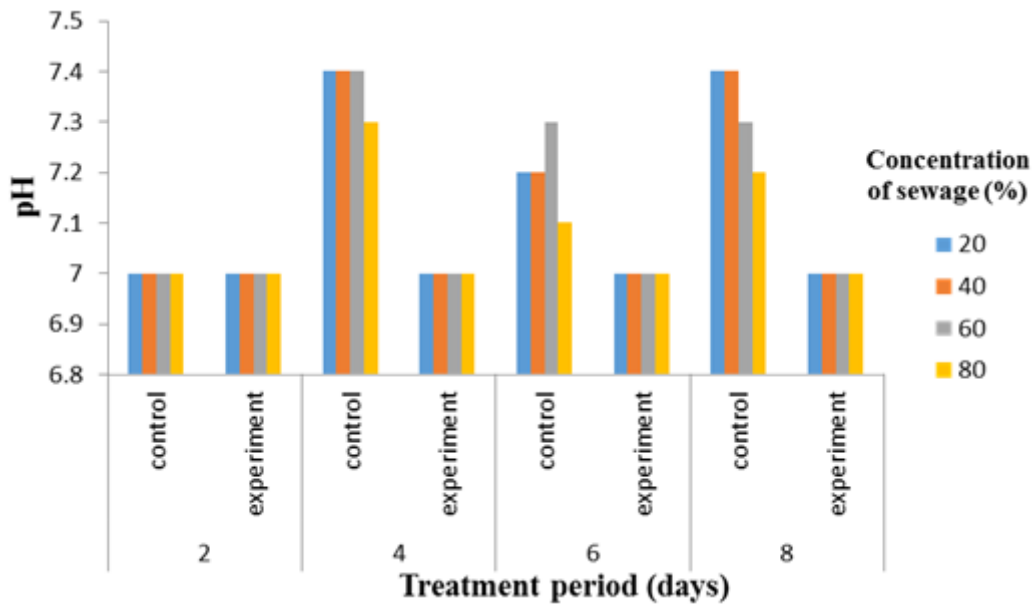


Fig-2. Changes in the levels of total solids (mg/L) in sewage after treatment with *P. pardalis*

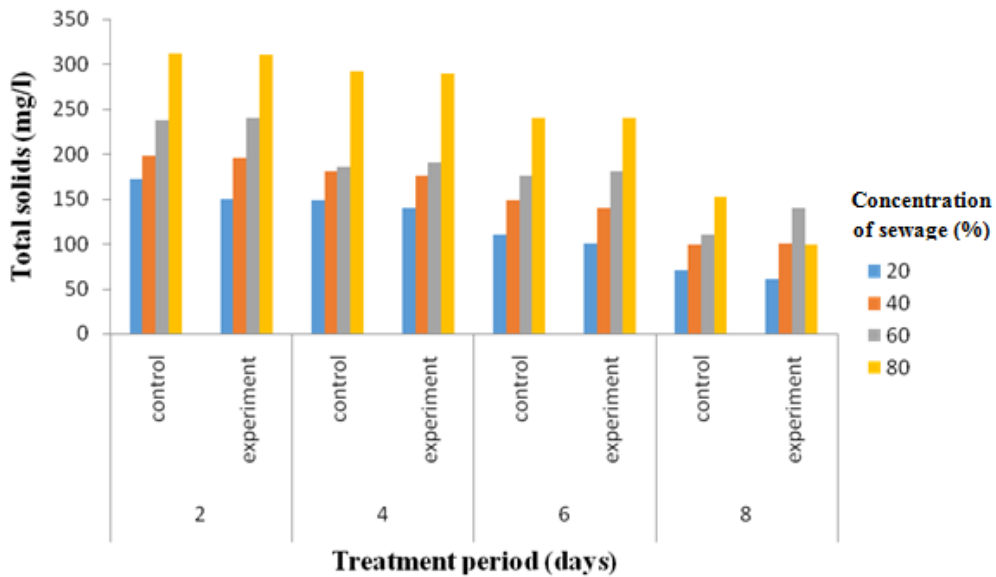


Fig-3. Changes in the levels of total dissolved solids (mg/L) in sewage after treatment with *P. pardalis*

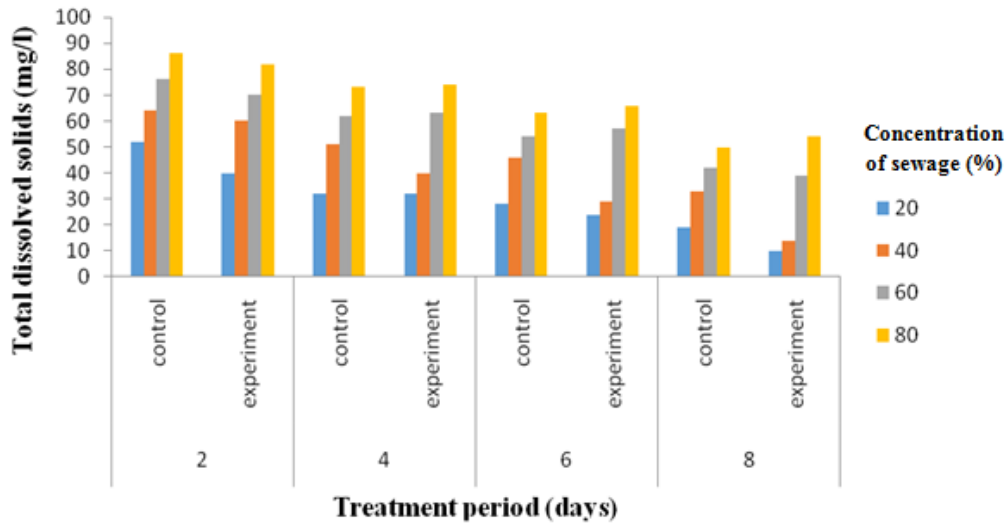


Fig-4. Changes in the levels of total suspended solids (mg/L) in sewage after treatment with *P. pardalis*

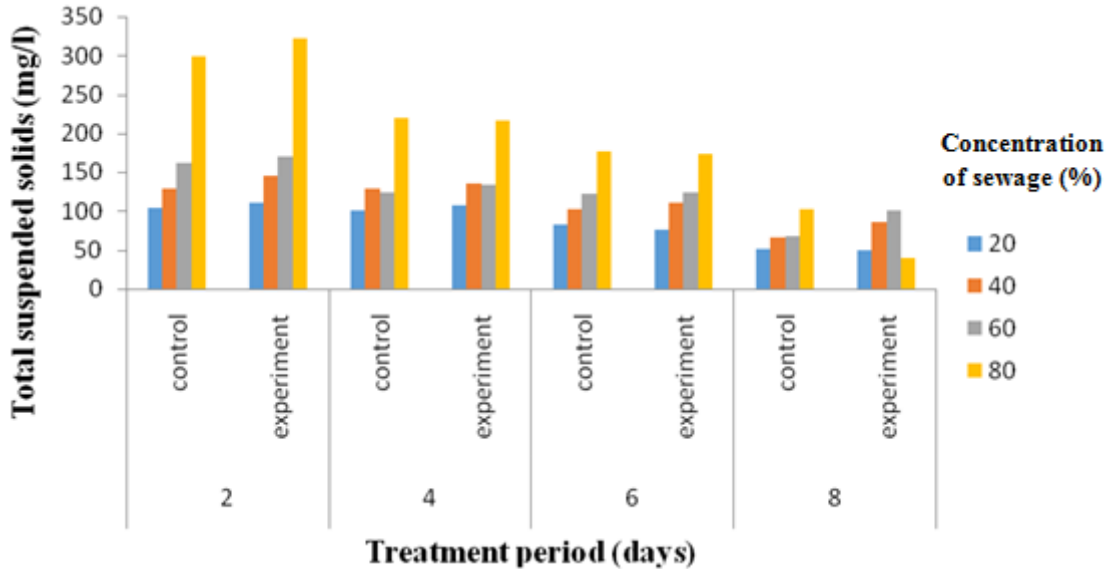


Fig-5. Changes in the levels of BOD₃30°C in sewage after treatment with *P. pardalis*

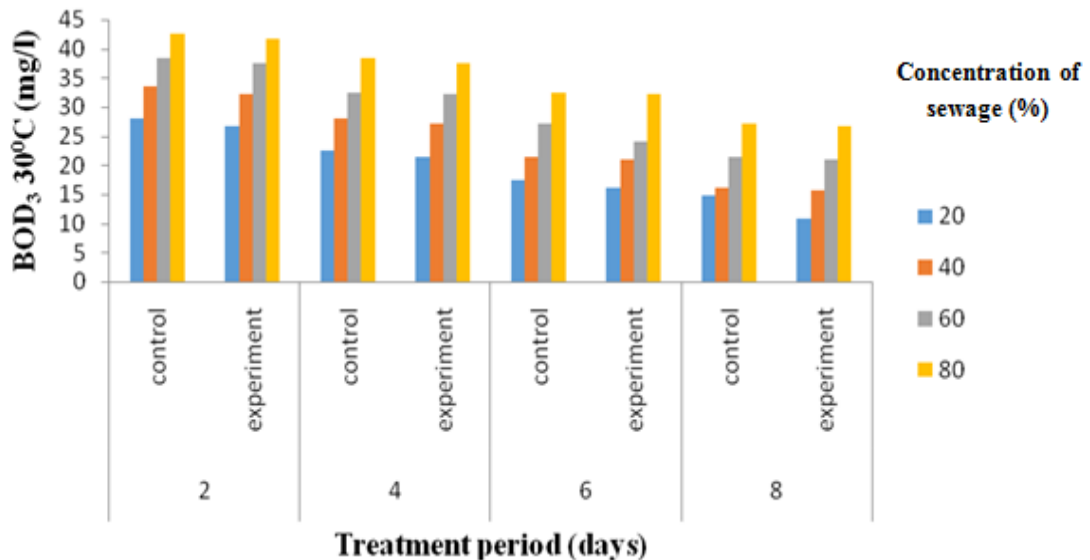


Fig-6. Changes in the levels of phosphates in sewage after treatment with *P. pardalis*

