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Effects of Different Zinc Concentrations on Culture Growth of *Spirulina platensis* and Its Production of Zinc Enriched as Superfood

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ABSTRACT

With its high protein, vitamin and mineral content, *Spirulina platensis* (SP) is the most widely used microalgae as a food supplement and the most cultivated microalgae for this purpose. Zinc is a regulatory microelement that is incorporated into the structure of many proteins in the cell and is particularly deficient in cereal-based societies. Due to the high adaptability of SP to environments with high metal concentrations and its high capacity to secrete substances called phytochelatin and metal-binding capacity, in this study zinc-enriched SP (ZnSP) was produced by binding metals to SP by organic means. For this purpose, modified media with 4 different Zn concentrations were prepared and SP was cultured in these media. Optical density, chlorophyll-a, phycobiliprotein and dry cell weight analyses were performed to monitor the culture. During the culture period, biomass and filtered culture medium were collected from logarithmic and stationary stages and Zn analyses were performed. The most suitable culture medium and growth conditions were determined to obtain Zn-enriched SP. 338.4 mg kg^{-1} Zn was measured in SP biomass grown in Zn-3 medium containing 8 mg L^{-1} Zn. It may be possible to obtain Zn-enriched SP in this medium and under the specified culture conditions, and even this ratio can be increased by adding Zn to the culture medium after the logarithmic stage.

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Introduction

Zinc is the second most abundant trace element after iron and is associated with many enzymes in the human body. It is involved in many metabolic processes such as DNA, RNA and protein synthesis, gene expression, synthesis and regulation of enzymes, release and storage of hormones, growth and development, healing of injuries, reception and transmission of impulses, regulation of central nervous system activities (Tapiero & Tew, 2003). It also regulates the functioning of the immune system and is important for the development and function of immune cells (Hojyo & Fukada, 2016). In addition, its antioxidant properties help to protect cells from oxidative damage caused by free radicals. Zinc deficiency has been shown to stimulate and accelerate apoptosis (Formigari et al., 2007).

Zinc is mainly found in red meat, chicken, fish, seafood, all cereals and dairy products. The bioavailability of zinc varies depending on the source and cooking technique (Lim et al., 2013). Zinc deficiency is common in our country, where we tend to eat a cereal-based diet. The phytate molecule in cereals binds zinc and reduces its bioavailability (Gupta et al., 2013). Soya protein, which is often used for its high protein content, contains a significant amount of phytate, which has a negative effect on zinc absorption. Amino acids, especially cysteine, histidine and methionine, increase zinc absorption (Hall & King, 2023). The amount of protein in the diet is positively correlated with zinc absorption also.

The World Health Organization (WHO) reports that 800.000 people die each year from zinc deficiency. 450.000 of these are children under the age of five (Das & Green, 2013). Approximately 2 billion people worldwide are affected by zinc deficiency (Yokokawa et al., 2023). The United Nations (United Nations System Standing Committee on Nutrition, 2004) reported that more than half of the world's population is deficient in micronutrients and that the majority of this group are children and women. Zinc deficiency is observed in about 1/3 of the world's population (Walsh et al., 1994). The main reason for zinc deficiency is that the diet does not include foods of animal origin, which are rich in these minerals, and high amounts

of cereals, which are rich in phytate, which binds phosphate and other minerals. In our country, 37 per cent of the daily energy requirement is provided by cereals and cereal products, while only 6 per cent is provided by meat and fish (Güzelcan et al., 2011). The United Nations Food and Agriculture Organisation (FAO) has stated that cereal crops grown on more than 50% of the world's soils are deficient in zinc (FAO, 2023). In our country, about 50% of soils are deficient in zinc (Kınacı et al., 2010). Iron deficiency was found in 49.7%, zinc deficiency in 18.9% and subclinical vitamin A deficiency in 14.7% of 334 children included in the study (Ekemen at al., 2018). Zinc, which is very effective in the growth and development of children and in human health, should be supplied to the body through a balanced diet and its deficiency should be corrected.

Spirulina platensis (SP), a member of the Oscillatoriaceae family, is being touted as a functional food with a protein content of up to 50-70%, high levels of polysaccharides, essential fatty acids and amino acids, vitamins (vitamin B types, vitamin E, vitamin A precursors), minerals and antioxidant pigments (phycobiliproteins, carotenoids, chlorophyll-a). SP is the most cultivated microalgae in the world and is hailed as the superfood of the future. The Global Spirulina Market Size is estimated to be USD 480 Million in 2021 and is projected to reach a market size of USD 1.1 Billion by 2030, at a CAGR (compound annual growth rate) of 10.4% from 2022 to 2030 (Spirulina Market Size, 2022).

Microalgae have the potential to be used in the removal of heavy metals because of their high bioremediation capacity. The phytochelatins they produce and the exopolysaccharides they secrete bind the metal ions in the environment, thus removing these substances from the environment. The most used medium for the culture of SP is the Zarrouk culture medium. This medium contains 0.05 mg L^{-1} Zn. According to FoodData central data 2 mg zinc 100 g^{-1} *Spirulina*. On the other hand, it is known that SP can survive in high concentrations of zinc and to adsorb zinc (Zhou et al., 2018).

The high ratio of protein and essential amino acids (methionine (14 mg g^{-1}), cysteine (7 mg g^{-1}), histidine (10 mg g^{-1})) and Vitamin B6 (8 µg g^{-1}) of *Spirulina* increases the bioavailability of zinc (Liestianty et al., 2019). In addition, there is no phytate molecule in SP, which is known to bind divalent cations and reduce their bioavailability (Ebid et al., 2022). These properties suggest the cultivation of SP in a high zinc medium and the production of zinc-enriched spirulina (ZnSP). People who consume such a product will benefit from the protein, vitamins and antioxidant pigments of SP and meet their zinc requirements.

Based on this information and considerations, the aim of this study was to optimize the culture of SP in Zarrouk media modified in terms of Zn concentration and to obtain ZnSP biomass. For this purpose, SP was cultured in media with four different Zn concentrations and culture growth parameters were monitored twice a week. Zn analyses of both culture medium and biomass were performed in logarithmic and stationary phases. The culture medium with the highest Zn concentration (Zn-3) was found to be the optimum growth medium for obtaining ZnSP.

Materials and Methods

Materials

For stock culture, SP was obtained from MAKUMACC with strain number MAKUMACC-093. SP was cultivated in the Zarrouk medium (Table 1) (Zarrouk, 1966). In 5 L flasks, 4 L medium was added and autoclaved at 121°C for 30 min. After cooling, SP was inoculated in the flasks which kept in an incubator at 30 °C, 12:12 photoperiod, for aeration gas flow rate was adjusted to 1Lmin^{-1}, pH value was 8.7-9 and a light intensity of 200 µmol photons m^{-2}s^{-1} of fluorescent lights. All process was carried out under sterile conditions.

Methods

Zinc Modifications and Culturing Methods

In this study, Zarrouk medium was determined as the control group, and also media with 3 different zinc concentrations were prepared. As totally 4 different culture media were created (Control, Zn-1, Zn-2, Zn-3) and SP was occulated to each medium with the close initial OD values (Table 2). Cultures in all media were sustained for 37 days and culture growth parameters were detected every other day.

Detecting of Culture Growth Parameters

For the determination of the cell density, the optical density (OD) values were measured at 680 and 750 nm in the spectrophotometer (Shimadzu UV-1650). 1 mL of sample from each culture medium was taken homogenously and placed in the cuvette and measured in 3 replicates (Santos-Ballardo et al., 2015).

One of the parameters that gives information about the culture status in the growth process of the culture is the Dry Cell Weight (DCW, mg L^{-1}) in the culture. For this, on the same days when OD is determined, 40 mL of samples were taken from the cultures, filtered with a glass fiber filter (Whatman GF/C, 1.2 mm, UK), dried in an oven at 40°C and determined in the following equation (1) (Vonshak, 1986).

Table 1. Recipe of culture media (Zarrouk. 1966).

Zarrouk culture medium	
Nutrients	Amount (gL^{-1})
NaCl	1.0
$CaCl_2$	0.04
$NaNO_3$	2.5
EDTA (Na)	0.08
K_2SO_4	1.0
$MgSO_4.7H_2O$	0.2
$NaHCO_3$	16.8
K_2HPO_4	0.5
$FeSO_4.7H_2O$	0.01 mL^B
Trace metal mix^C	1 ml L^{-1}

B: 10 g of $FeSO_4.7H_2O$ in 100 ml 1N HCl; C: $ZnSO_4.7H_2O$, 0.222 mg L^{-1}; $CuSO_4.5H_2O$, 0.079 mg L^{-1}; MoO_3, 0.015 mg L^{-1}; H_3BO_3, 2.88 mg L^{-1}; $MnCl_2.4H_2O$, 1.81 mg L^{-1}

Table 2. Zinc concentrations and initial optical density values of all culture media.

Media	Control	Zn-1	Zn-2	Zn-3
Zn Concentrations (mgL^{-1})	0.05	2	4	8
Initial OD_{680} values (abs)	0.391	0.405	0.377	0.356
Initial OD_{750} values (abs)	0.290	0.314	0.293	0.285

DCW= FFW- FIW (1)

DCW: Dry cell weight (DCW)
FFW: Filter final weight
FIW: Filter initial weight (g L–1)

Spesific Growth Rate (SGR, μ), Doubling Time (Dt, day), and Biomass Productivity (BP, g $L^{-1}day^{-1}$) values were calculated separately for each type established. The calculations of these values were done as follows.

The SGR of SP was calculated using Equation (2) (Chia et al., 2013).

$$\mu = \frac{ln\left(\frac{x_1}{x_2}\right)}{t_2 - t_1} \quad (2)$$

Where μ is specific growth rate (day^{-1}) and X_1 and X_2 are the OD_{750} at time t_1 and t_2 (day), respectively.

Dt (Godoy-Hernández et al. 2006) and BP (Hempel et al. 2012) were calculated with the following equations.

$$Dt = 0.6931 / \mu \quad (3)$$

$$BP\ (g\ L^{-1}\ day^{-1}) = (X_2 - X_1) / (t_2 - t_1) \quad (4)$$

X_1 and X_2 are the dry weight of biomass at times t_1 (culture starting time) and t_2 (culture finishing time).

Chlorophyll a (chl-a) amount was detected according to (Lichtenthaller, 1987 and Wellburn, 1994) for observing culture growth. 1 mL culture sample was centrifuged, the supernatant was discarded. 2 mL methanol (99%) was added onto the pellet. It was incubated in a hot water bath at 60°C for 30 minutes. Then 10 min. incubated on ice and at 13000 rpm for 5 min. it was centrifuged. After centrifugation, the pellet should be completely white, if not, the extraction process was repeated. Methanol was used as blank, extraction was measured in the spectrophotometer at 665.2, 652.4 and 470 nm. Calculation was made using the formula below.

$$\text{Chlorophyll a (chl-a)} = 16.72(A_{665}.2) - 9.16(A_{652.4}) \quad (5)$$

To determine the amount of phycobiliprotein concentration, phycocyanin, phycoerythrin and allophycocyanin amounts were measured. For extraction 10 mM sodium phosphate buffer (pH 7.0) was used. All other processes were made according to Arashiro et al., (2020). The optical density of the extracts was measured in a spectrophotometer (Shimadzu UV-1280, Japan), 280, 562, 615 and 652 nm. The amounts of phycocyanin (PC), allophycocyanin (APC) and phycoerythrin (PE) were calculated according to formulas.

$$PC\ (mg / mL) = [A_{615} - (0.474 \times A_{652})] / 5.34 \quad (6)$$
$$APC\ (mg / mL) = [A_{652} - (0.208 \times A_{615})] / 5.09 \quad (7)$$
$$PE(mg/mL) = [A_{562} - (2.41 \times PC) - (0.849 \times APC)] / 9.62 \quad (8)$$

Zn Measurement of Culture Media and Microalgae Biomass

To detect the Zn amount in biomass and in the culture media, biomass and also culture medium were harvested at two different times during the culture period. The first at the logarithmic stage of culture (16th day) and the second at the stationary stage of culture (37th day). Zn amounts of microalgae biomass and filtered culture media were determined by Perkin Elmer ICPOES Optima 8000. Approximately, 0.5 g dried samples were dissolved in 9 mL nitric acid and 3 mL hydrochloric acid mixture (HNO_3: HCl=3:1,v/v). After preliminary digestion, the mixture was placed in a microwave digestion system (Milestone Stard-D) for further digestion. The oven temperature was 110 °C for 15 min. Then, the sample was cooled to room temperature and measured by ICPOES.

Statistical Analysis

The data consisted of the means and standard deviations of at least three replicates. The obtained data were statistically examined at 0.05 using the least significant difference (LSD).

Results and Discussion

Culture Growth Parameters

In microalgae culture studies, regular monitoring of culture growth parameters from the beginning to the end of the culture is of great importance in terms of determining the general condition of the culture and the harvest time. For this reason, optical density, dry cell weight, chl-a and total phycobiliprotein values were measured every other day during the culture period and culture growth curves were prepared with these data (Figure 3, 4, 5, 6 and 7).

Cultures in all media were started with equal initial OD values and optical density values of all culture media were measured every other day. It was observed that SP showed growth in all media but according to Figure 3 and 4, in general maximum OD values were recorded in Zn-3 medium. Maximum OD value is recorded as 1.38 (680 nm) and as 1.19 (750 nm) in Zn-3 medium on 30th day of culture. For the biomass that obtained from other media have lower OD values than the Zn-3 medium. This shows that increasing of Zn content in the culture medium causes a huge optical density. However, this result may be due to the intensive culture caused by long filaments, as shown in Figure2. If the morphological characteristics are examined in detail, it can be seen that the longest filament is in SP in Zn-3 medium.

When examining the DCW results, it can be seen that the highest dry cell weight was measured in the Zn-1 medium (0.00126 g mL^{-1}) on day 27 (Figure 5). This was followed by Zn-3, Zn-2 and control groups on the same day. This shows that the Zn-3 medium with a high OD value does not have a high DCW value at the same time. In fact, the high OD value of Zn-3 medium is due to long filaments.

With using DCW values, BP and Dt values were estimated (Table 3). The lowest BP value was recorded in the Zn-3 medium. However, the highest OD values were also recorded in this medium. These results show that long filaments (Figure 2) in this medium caused high OD values, but due to high Zn concentrations there was a stress on the microalgae so that the same high biomass productivity values were not obtained in this medium. It is also known that filament elongation is related to abiotic stress in the culture medium (Faluweki & Lucas, 2022) and it was clear from these results that a high Zn concentration created a stressful situation for microalgae and there was no high biomass production

Table 3. The results of estimation of Spesific Growth Rate (SGR), Doubling Time (DT) and Biomass Productivity (BP) values of all culture media.

Media	Control	Zn-1	Zn-2	Zn-3
SGR (day^{-1})	0.0458±0.001	0.0253±0.0003	0.0256±0.002	0.0385±0.01
DT (day)	15.1172±0.0001	27.3429±0.001	27.0310±0.01	17.9921±0.001
BP ($gL^{-1}day^{-1}$)	0.0308±0.0021	0.0310±0.002	0.0490±0.001	0.0276±0.001

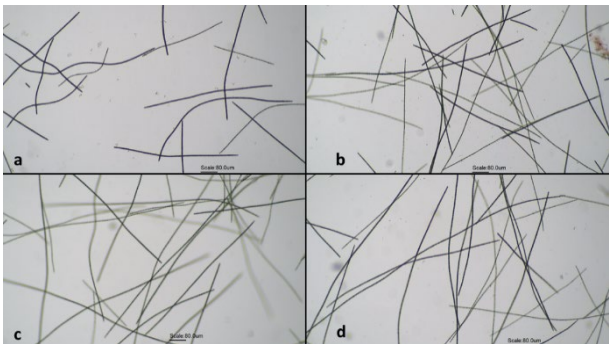

Figure 1. Light micrographs (10X) of *Spirulina platensis* in the in four different culture media during the cultivation period (Scale bar: 80.0 um). a: Zn Control, b: Zn-1; c: Zn-2; d: Zn-3.

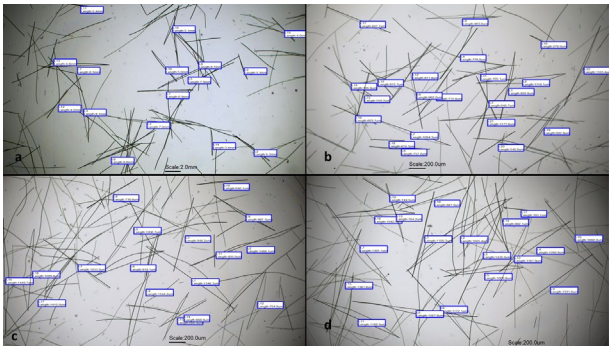

Figure 2. Filament measurements (4X) of *Spirulina platensis* in in four different culture media during the cultivation period. a: Zn Control, b: Zn-1; c: Zn-2; d: Zn-3.

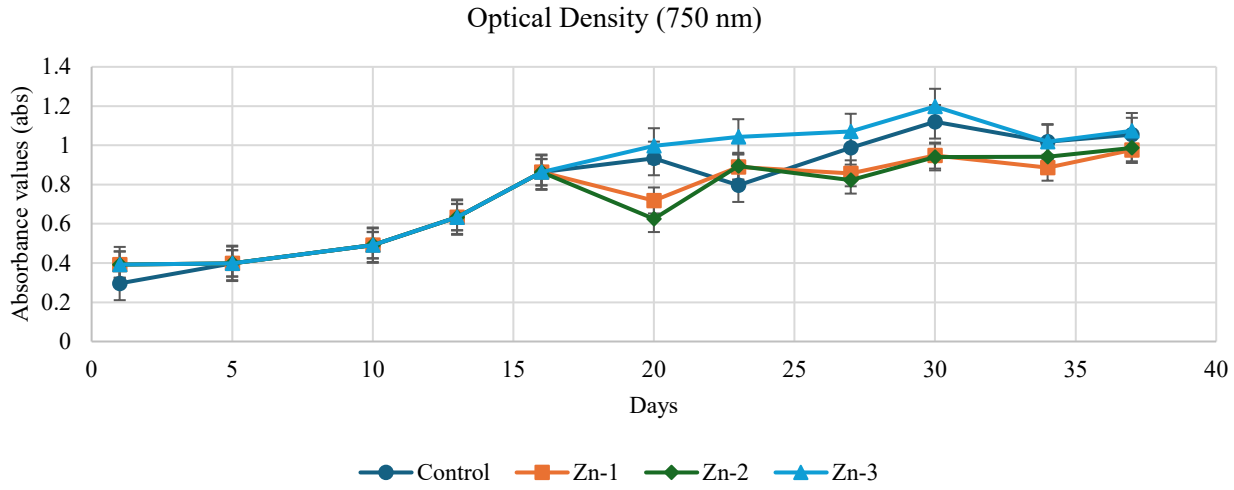

Figure 3. OD_{750} of *Spirulina platensis* in four different culture media during the cultivation period. Error bars represent the standard deviation among the three replicates of each culture condition (n=3, $p<0.05$)

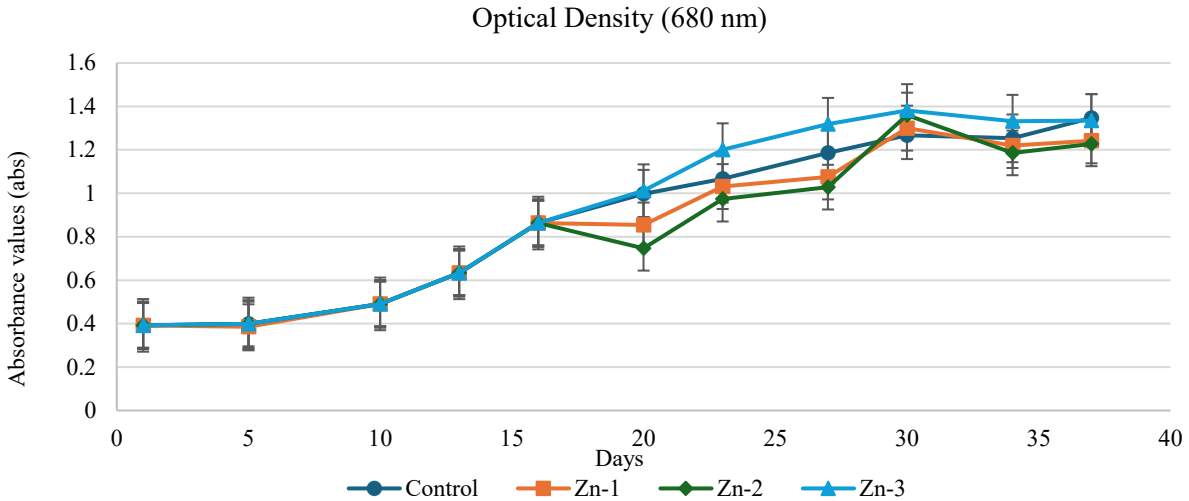

Figure 4. OD_{680} of *Spirulina platensis* in four different culture media during the cultivation period. Error bars represent the standard deviation among the three replicates of each culture condition (n=3, $p<0.05$).

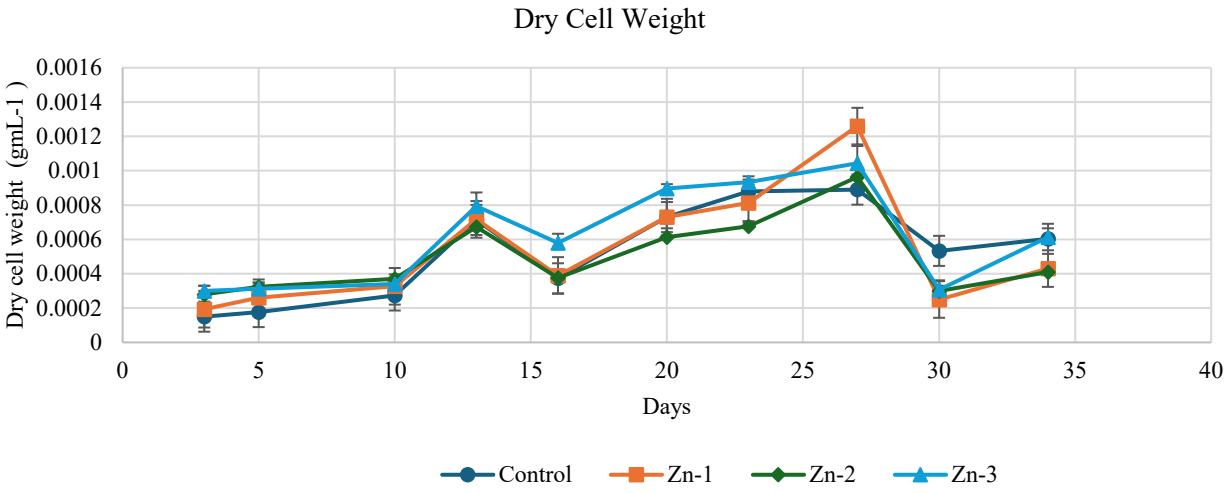

Figure 5. Dry cell weight (DCW) of *Spirulina platensis* in four different culture media during the cultivation period. Error bars represent the standard deviation among the three replicates of each culture condition (n=3, $p<0.05$).

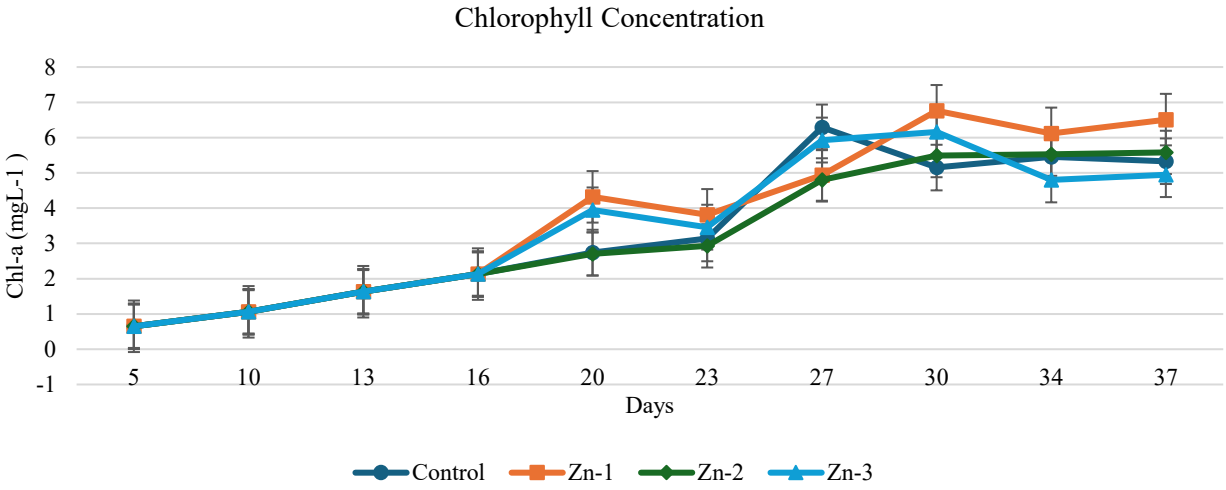

Figure 6. Chlorophyll-a (Chl-a) values of *Spirulina platensis* in four different culture media during the cultivation period. Error bars represent the standard deviation among the three replicates of each culture condition (n=3, $p<0.05$).

BP results show that for obtaining the high biomass production, the best medium is Zn-2 medium. Because the highest BP value (0.049 g L^{-1} day^{-1}) was recorded in this medium. On the other hand, SGRs were estimated with the OD values and showed in Table 3. The highest SGR value (0.0458 day^{-1}) and the shortest DT (15.117 day) were found in the control medium, which is the most commonly used medium in SP culture. This result is quite normal as this is an optimized medium. The aim of this study was not to monitor the survival of SP at optimum Zn concentrations, but to investigate its viability at increasing Zn concentration, to optimize this medium and culture conditions and to obtain ZnSP biomass. The second highest SGR (0.0385 day^{-1}) was observed in Zn-3. This result shows that SP can survive in culture medium with high Zn concentration, but the BP value of this medium does not increase in the same way. Considering the commercial production process of the ZnSP, high biomass productivity is required. Therefore, Zn-2 medium, which is the medium with both high BP and SGR, seems to be more suitable for commercial production. Or, with the different application suggestions we have given below, this problem can be overcome and SP biomass containing more Zn can be obtained.

From the analysis of Figure 6 it can be seen that the highest chl-a value (6.12 mgL^{-1}) was in the Zn-1 medium on day 30. In fact, SP tolerated all Zn concentrations and adapted to all four media. However, the fact that the amount of chl-a was higher in the Zn-1 medium shows that SP lived in a healthier way in this medium. On the same day, the amount of phycobiliprotein was lowest (0.040 mgL^{-1}) in the Zn-1 medium and highest (0.065 mgL^{-1}) in the Zn-3 medium. On the 30th day it was observed that the phycobiliprotein value of Zn-3 medium was higher than the other media. This indicates that SP was stressed in the Zn-3 medium but adapted to this medium with high phycobiliproteins. It is known that the amount of phycobiliprotein in cyanobacteria increases under stress conditions (Carnicas et al., 1999). As can be seen from the above results, the reason for the higher OD value of the Zn-3 medium may be the high phycobiliprotein value as well as its long filaments.

Zn content of Microalgae Biomass and Filtrated Culture Media

As the Zn concentration in the culture medium increases, the amount of Zn in the SP biomass also increases (Figure 8). It can be seen that SP can easily survive in the medium containing higher concentrations of Zn compared to the control medium and can absorb Zn into the biomass. The logarithmic stage and the stationary stage were sampled during the culture period and it was observed that the amount of Zn in the biomass taken from the stationary stage was high in 4 different media. The highest Zn concentration (338.44 $mgkg^{-1}$) was reached in the biomass taken from the stationary stage in Zn-3 medium, and a 33.8-fold increase was observed compared to the biomass taken from the control group. Similarly, Zhou et al., (2018) reported a 27.2-fold increase in the total zinc content of the medium containing 4 mgL^{-1} Zn compared to the control. In the same medium, the highest amount of Zn was observed in the biomass (211.33 $mgkg^{-1}$) taken at the logarithmic stage. Analyzing the graphs of the culture growth parameters, it can be seen that the Zn concentration in the Zn-3 medium is not high for SP and SP tolerates this high Zn concentration by showing healthy growth. These results show that our modified Zn-3 medium is a very suitable medium for obtaining ZnSP and that our physical conditions are designed in such a way as to have a positive effect on growth. On the other hand, some studies indicate that increasing the amount of Zn in the culture medium does not always increase the amount of Zn in the biomass, and sometimes even high Zn concentration inhibits Zn accumulation (Zdziebłowska et al., 2024). Ghafari et al., (2015) explained that much higher Zn concentrations increased cell proliferation and lipid degradation in their study with different microalgal species.

The Zn content in the SP biomass was analyzed as well as the Zn content in the filtered culture medium remaining after removal of the biomass, and the results are shown in Figure 9.

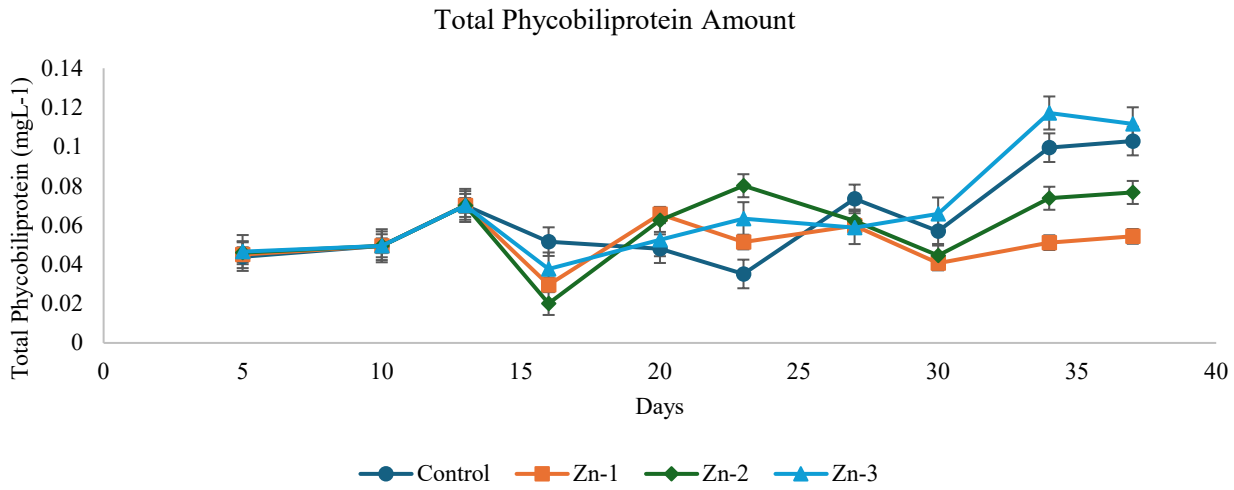

Figure 7. Total phycobiliprotein values of *Spirulina platensis* in four different culture media during the cultivation period. Error bars represent the standard deviation among the three replicates of each culture condition (n=3, $p<0.05$).

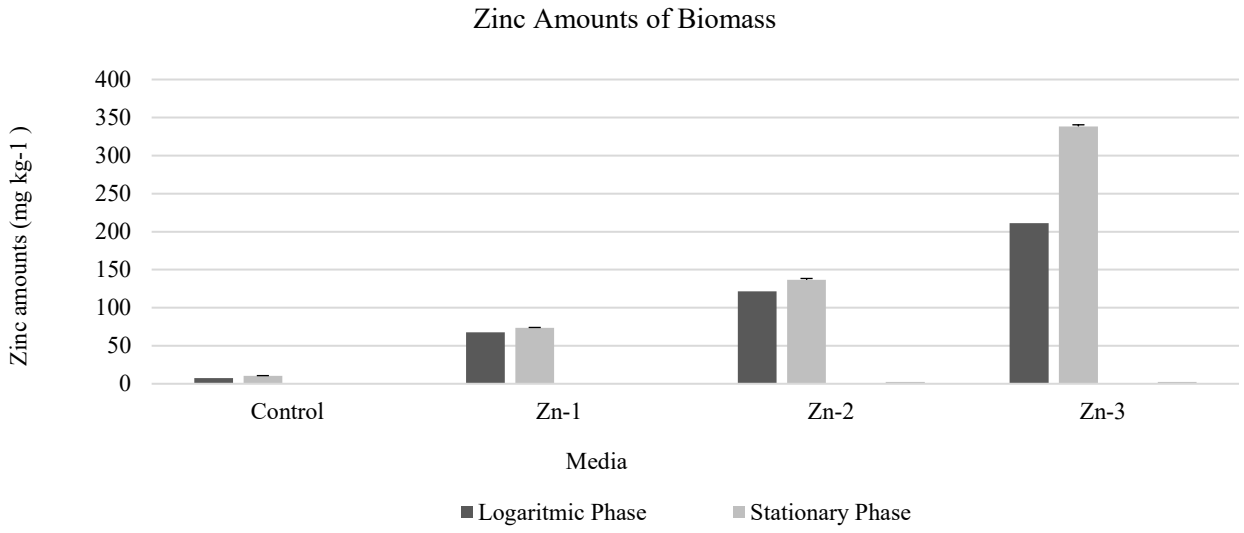

Figure 8. Zinc amounts of *Spirulina platensis* biomass were obtained from four different culture media and two different culture phases. Error bars represent the standard deviation among the three replicates of each culture condition (n=3, $p<0.05$)

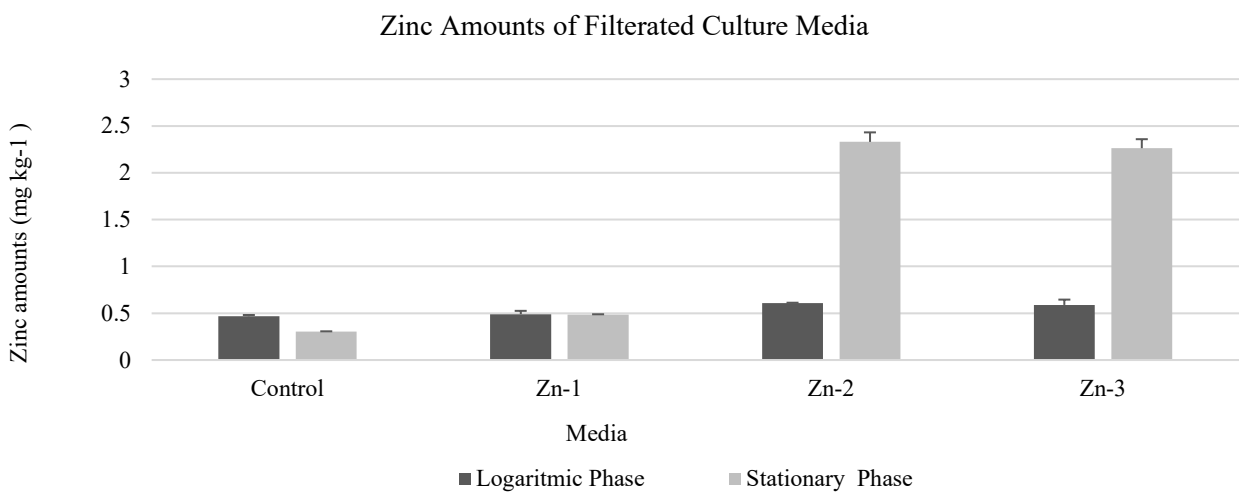

Figure 9. Zinc amounts of filtrated culture media were obtained from four different culture media and two different culture phases. Error bars represent the standard deviation among the three replicates of each culture condition (n=3, $p<0.05$).

In the control and Zn-1 media, since SP was able to completely consume the available Zn, there were no major differences in the amount of Zn between the logarithmic phase and the stationary phase. For example, the amount of Zn in the filtered culture medium obtained from the Zn-1 medium was 0.49 $mgkg^{-1}$ in the logarithmic phase and 0.48 $mgkg^{-1}$ in the stationary phase. However, since Zn concentrations were higher in Zn-2 and Zn-3 media, there was a difference between logarithmic and stationary phase in terms of Zn concentration. This situation shows that Zn in the culture medium can be taken up as much as it can be taken up by the SP, and increasing the amount of Zn further will not increase the amount of Zn in the SP and the cells will not be able to take it up. The results in Figure 9 also show that the Zn concentrations we modified in our study are very suitable for our purpose, and increasing the amount of Zn further may stress the cell. Furthermore, when analyzing the SGR and BP values (Table 3), it can be seen that the highest SGR value belongs to the control medium (0.045 day^{-1}), followed by the Zn-3 medium (0.038 day^{-1}). The fact that the Zn-3 medium with the highest Zn concentration has an SGR value close to that of the control medium indicates that this medium can be used to obtain ZnSP biomass.

On the other hand, Zn-3 medium had the lowest BP value (0.027 g L^{-1} day^{-1}), suggesting that this medium cannot be used for this purpose. In Zn-3 medium, the cells were stressed, so they started to divide and elongate, and as a result the highest OD values were recorded in this medium due to elongated filaments (Figs 3 and 4). Again, the high levels of phycobiliprotein, which has a high antioxidant capacity, in the Zn-3 medium, especially in the stationary phase, indicate that the cells were stressed (Figure 7).

At this point it is necessary to consider how the metals can be used in the cell. Cyanobacteria take up metals either by binding them to functional groups in the cell membrane or by accumulating them in the interior of the cell (Pane et al., 2008.). High levels of metal uptake are associated with the production of substances called phytochelatins, which are responsible for detoxifying heavy metals (Pawlik-Skowronska, 2001). These molecules are present in the cell as metal-binding proteins and provide resistance to high metal concentrations, especially in cyanobacteria (Harada et al., 2004). In fact, it is necessary to measure the phytochelatin concentration of the cell to make a more realistic inference as to which medium should be chosen.

When all these data are evaluated together, it can be seen that Zn-3 medium can be used for the production of ZnSP, but low biomass productivity will be encountered. To overcome this problem, starting the culture with a high OD value can shorten the culture period and more biomass can be obtained in a shorter time. Another method is to add Zn to the culture medium after the logarithmic phase. This practice also allows the cells to be exposed to a high concentration of Zn for a shorter period of time, thus preventing the cells from experiencing prolonged stress. Zinicovscaia et al., (2021) emphasised that the time of Zn addition to the culture is important in determining tolerance to metal ions. In such applications, the desired ZnSP production can be achieved with higher biomass efficiency. It is of great importance to carry out ZnSP, known as superfood, organically by biological processes and to determine the most suitable and efficient modified culture media and culture conditions.

In fact, there is not much data in the literature on the metal bioaccumulation capacity of microalgae and the factors that influence it. It is almost impossible to draw complete and accurate conclusions. This is because the rate of accumulation of a metal in a microalgae cell varies depending on the type of microalgae, the culture conditions, the concentration of the metal and other metals in the culture medium. For this reason, the determination of the bioaccumulation rates of microalgae in culture media containing the most deficient metal in society and at different concentrations of this metal is important both to fill the gap in the literature and to obtain a commercial product. Our study has been a study that serves this field and determines the most suitable Zn concentration and culture conditions for the production of ZnSP.

Conclusion

Determining which modified culture medium to use and under what conditions to produce ZnSP is a complex process with many parameters to monitor. The Zn binding capacity of SP varies depending on the concentration of Zn and other metals in the culture medium, the culture conditions and the time of addition of Zn to the culture medium. In this study, we added Zn at the beginning of the culture because we wanted Zn not only to bind to the cell membrane surface, but also to enter the cell and bind to proteins through organic processes. In our study designed for this purpose, we were able to achieve 338.44 mg kg^{-1} Zn in Zn-3 medium, and most of this Zn is bound to metalloproteins in the cell due to prolonged treatment with the cell. In future studies, higher concentrations of Zn can be tested, and higher levels of Zn-bound SP production can be attempted. Considering that commercially produced ZnSP should provide 1/3 of the daily intake of 10 mg Zn with 3 tablets of 1 mg, the Zn-enriched SP to be produced should have a ratio of 1000 mg kg^{-1}.

Declarations

Author Contribution Statement

Füsun Akgül: Data collection, investigation, writing the original draft.

Rıza Akgül: Methodology, review and editing.

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Conflict of Interest

The authors declare no conflict of interest.

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