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Impact of Metallic Nanoparticles on the Nutritional Values of *Spirulina*

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ABSTRACT

Spirulina has high nutritional values and anti-oxidative properties. It is a staple diet due to its easy cultivation and greater nutritional values in biological macromolecules (proteins, lipids, and carbohydrates), pigments (chlorophyll, carotenoids, phycobiliproteins) vitamins, minerals, phenolic compounds, and amino acids. *Spirulina* also has been used as a nutraceutical to treat numerous diseases and disorders due to its promising therapeutic values. However, extensive anthropogenic activities cause the discharge of metals and metallic nanoparticles into the environment that might cause toxicity to marine and freshwater microalgae due to bioaccumulation. The presence of metals in the environment beyond the normal range does not only affect the growth but also the nutritional values of microalgae. The nutritional properties and usage of *Spirulina* along with the harmful effects of metals and metallic nanoparticles on *Spirulina* are highlighted and summarized in this paper.

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1 Introduction

Spirulina has been consumed for nearly centuries and is the most consumed microalgae species. World Health Organization (WHO) coined *Spirulina* as a "superfood" concerning its higher nutritional values and anti-oxidative properties (Liang et al. 2021). It is among one of the 35 wild species that are commonly found in lakes with extreme alkalinity (Raj et al. 2020). *Spirulina platensis* (*Arthrospira platensis*), *S. maxima* (*Arthrospira maxima*), and *S. fusiformis* (*Arthrospira fusiformis*) are widely studied due to their edible properties and nutritional values in addition to their promising therapeutic values (Beheshtipour et al. 2012; Michael et al. 2018). The Mexican population had been using *S. platensis* as a source of food since the Aztec civilization (Farrar 1966). It is majorly commercialized as a staple diet due to its greater nutritional value, easy cultivation, and low cost (Molino et al. 2018). However, recent anthropogenic effects such as urbanization, and current industrial and agricultural activities have led to the contamination of soil and aquatic environments (Ekubo and Abowei 2011). The massive industrial usage of engineered metal nanoparticles (MNPs) such as zinc, silver, titanium, and copper nanoparticles in industrial applications results in the intentional and accidental release of these nanoparticles into the aquatic environment (Praveena et al. 2010) which can affect the growth and the nutritional values of microalgae growing in the polluted water bodies through physiological and biochemical alterations (Navarro et al. 2008; Morais et al. 2009). This review presents the nutritional properties and usage of *Spirulina*, along with the effects of metals and metal nanoparticles on *Spirulina*.

2 Nutritional Values osf *Spirulina*

Spirulina species are highly nutritious and contain a significant amount of proteins, lipids, carbohydrates, beta-carotene, vitamins, amino acids, and minerals (Khan et al. 2005). *Spirulina* species generally contain 55.0 - 70.0% proteins, 15.0 - 25.0% carbohydrates, 5.0 - 6.0% lipids, 6.0 - 13.0% nucleic acids, and 22.0 - 48.0% minerals (Kulshreshtha et al. 2008). The major percentage constituent level of protein in *Spirulina* is the main reason for it to be chosen as a food source. Carbohydrate content usually acts as a secondary role when the microalga is used as protein-rich food (Becker 2013). The structure of branched polysaccharides in *S. platensis* is similar to glycogen (Wan et al. 2016). Further, the lipid contents in this microalga average vary from 1.0 - 40.0% and, up to 85.0% of the dry weight under certain conditions. The lipids formed in *S. platensis* can be either saturated or unsaturated fatty acids (Becker 2013). *S. platensis* species also contain lots of vitamins such as a variety of vitamin B complexes which are B_1, B_2, B_3, B_6, B_9, and B_{12}, and minerals like calcium, copper, iron, magnesium, phosphate, sodium and zinc, and photosynthetic pigments (Houston 2002).

Microalga like *S. platensis* is rich in antioxidants due to the presence of various types of phytochemicals (Al-Dhabi and Valan Arasu 2016). These antioxidants are generated to nullify the oxidative stress (Xia et al. 2015) caused by reactive oxygen species (ROS) (Djearamane et al. 2020). The environment in that *S. platensis* thrives generally contributes to the such occurrence (Siddiqui and Prasad 2017; Hussein et al. 2019). Thus, with the accumulation of antioxidants, the effect of ROS is suppressed (Riss et al. 2007). Its properties as antioxidants are beneficial regardless of being used for humans or in animal feed (Wu et al. 2016). Two main varieties of antioxidants i.e. enzymatic antioxidants and non-enzymatic antioxidants are found in *S. platensis* (Carlsson 1978). The non-enzymatic antioxidants are the most significant ones consisting of chlorophyll, tocopherols, carotenoids, flavonoids, phycocyanin, phenolics, phycobiliproteins (PBPs), and polyunsaturated fatty acids (PUFAs) (Richmond 2004). Flavonoids known as phenolic acids are predominant in *Spirulina* with the ability to be antioxidants in suppressing various diseases such as infamous cancer and cardiovascular-related diseases as well as its scavenging ability towards free radicals (Wali et al. 2015). Further, phycocyanin, with phycoerythrin masks the pigmentation of chlorophyll (Karimi and Moradi 2015). These two photosynthetic pigments give *Spirulina* blue-green colour and hence it is known as the blue-green microalga (Desai and Sivakami 2004). Tables 1, 2, and 3 show the macronutrient composition of *Spirulina* and comparison with common human food sources. Based on Table 1, *S.platensis* has a well-balanced derived composition of various bioactive nutrients (Liang et al. 2020) varying from vitamins (inclusive of vitamin K, vitamin A, vitamin E, Thiamine B1, Riboflavin B2, Niacin B3, Vitamin B6, Vitamin B12, Provitamin A, Riboflavin B2, Folic Acid, Panthothenic acid and inositol (Koru et al. 2008)), minerals (8%), proteins (60-70%), phenolic compounds, γ-linolenic acid (GLA) (49%), phycocyanins (15%), and constituent of phytochemicals (Jaime et al. 2005; Stanic-Vucinic et al. 2018). In addition, it is also well known for its higher content of carotenoids (456.00 mg/100g of dry weight) (tetraterpenoids) including about 80% of beta-carotene and 20% of secondary carotenoids like xanthophyll which is typically higher than found in carrots (Siddiqui and Prasad 2017; Shao et al. 2019). Most importantly, *S. platensis* is rich in complete essential amino acids (EAA) and gamma linoleic acid (GLA) (Koyande et al. 2019). These attributes commercialized *S. platensis* as a nutritional food for human consumption despite the ongoing validation against the claims of its adversities which are yet to be claimed by scientific research. International and national quality standardization have been established to ensure safety and to avoid public health issues due to toxic blooms as well as other toxic issues from microalgae for safe consumption (Henrikson 2010).

Table 1 Comparison of macronutrients of *Spirulina* with the common human food sources

Sample	Average content in Percentage (% DW)			Reference(s)
	Proteins	Lipids	Carbohydrates	
Milk	26	28	38	(Becker 2013)
Rice	8	2	77	(Becker 2013)
Soybean	34-40	16-20	19-35	(Becker 2013)
Egg	45- 47	41	4	(Gouveia et al. 2008)
Cheese	30- 36	-	-	(Koru 2012)
Chicken	31	-	-	(Koru 2012)
Fish	15–22	-	-	(Koru 2012)
Baker's yeast	39	1	38	(Becker 2013)
S. fusiformis	62.3	8.2	19.3	(Rafiqul et al. 2003)
S. maxima	68-77	4-14	8-16	(Hoseini et al. 2013; Matassa et al. 2016)
S. platensis	46-74	6-13	8-25	(Hoseini et al. 2013; Matassa et al. 2016)

3 Therapeutic Effects of *Spirulina*

Various studies and trials have elucidated the therapeutic values of *S. platensis* (Al-Harbi 2008; Moor et al. 2017; Al-Qahtani and Binobead 2019; Shamsudin et al. 2019; Raj et al. 2020). *S. platensis* cell walls are made of liposaccharides that can induce the innate immune response through the toll-like receptor 4 (TLR-4) (McCarty et al. 2010). Although *S. platensis* is a Gram-negative bacterium, it does not exhibit an antagonistic reaction towards TLR-4 due to its nature as a cyanobacterium (Okuyama et al. 2017; DiNicolantonio et al. 2019). However, other Gram-negative bacteria such as *Salmonella* do not tolerate TLR-4 reactions (Lima et al. 2017).

The lack of cellulose in its walls enables digestibility up to 90%, which is relatively equivalent to the digestibility of casein (Mao et al. 2005). Even the absorption of iron is more than 50% higher than the conventional iron supplements. *S. platensis* richness in micronutrients puts it on the frontline for vegans and vegetarians especially when its iron content is thrice than found in meat as well as comparable with micronutrients found in milk (Bensehaila et al. 2015). Furthermore, it has been reported that *S. platensis* liposaccharides can inhibit the development and growth of tumors. Furthermore, C-phycocyanin inhibits the enzyme cyclooxygenase-2 (Cox-2) involved in the biosynthesis of prostaglandins (PGs). This Cox-2 is highly expressed in cancerous cells to promote PGs as inflammatory mediators (Fournier and Gordon 2000).

S. platensis is also an excellent free radical scavenger and immunological stimulant as proven by various *in vivo* and *in vitro* studies (Assaye et al. 2018). *Spirulina* is considered as a non-toxic dietary supplement and safe to be consumed at normal consumption levels (Raj et al. 2020). There is still a grey area in the possible reaction of *Spirulina* with therapeutic compounds or other dietary supplements (Hoseini et al. 2013), and some shortcomings are also associated with the consumption of *Spirulina*. Mazokopakis et al. (2008) reported that the consumption of *Spirulina* may induce headache, stomach ache, flushing of the face, and muscle pain effects. Severe side effects of hepatotoxicity and rhabdomyolysis are also reported due to the consumption of *Spirulina* tablets as dietary supplements (Iwasa et al. 2002). Papapetropoulos (2007) suggested that patients with phenylketonuria and autoimmune diseases should avoid taking *Spirulina*. Despite *S. platensis* is generally recognized as safe and highly nutritional with various constituents of nutrients and micronutrients, its intrinsic properties are fundamentally dependent on its cultivated conditions and environments (Belal and El-Hais 2012) which certainly affect the *S. platensis* nutrient bioavailability (Falquet and Hurni 1997).

4 Effects of Heavy Metals and Metallic Nanoparticles on *Spirulina*

The development of technology and nanotechnology (Castro-Bugallo et al 2014), human activity, and natural earth processes are leading to the release of metals and metallic nanoparticles (MNPs) into the environment (Tsao et al. 2011). Heavy metals are categorized as metals that have an atomic density higher than water or at least 4 grams per centimeter cube (Hawkes 1997). The presence of heavy metals amplified during the ore mining era which was the main source of accumulation of heavy metal in the environment besides the natural occurrence of heavy metals through leaching and the weathering effect of rocks filled with ore.

Studies on heavy metals mainly assessed the ecological risk. Most of the heavy metal accumulations are due to anthropogenic effects as the main contributors (Dubey 2021). Heavy metals are transported through water bodies like rivers and estuaries which act as a sink for these toxic matters to accumulate and influence the heavy metals accumulation in a particular area (Yap and Al-Mutairi 2021). The heavy metals flowing from upstream of rivers into the ocean accumulate in a huge amounts together before releasing into the ocean. Heavy metals are found in parent rock which dissipates and enters the environment through hydrological effects, weathering effects, and also by erosions (Mushtaq et al. 2020).

Recent industrialization with massive utilization MNPs such as silver, zinc, titanium, and copper NPs in numerous industrial and consumer products, and the subsequent release of these MNPs in the industrial and domestic wastewaters brings the threat of nano pollution of water bodies. Numerous studies have been conducted to analyze the effect of metals and metallic nanoparticles on microalgae, especially in the aspect of the growth rate and biochemical composition (Djearamane et al. 2019; Liang et al. 2021; Thenarasu et al. 2022). Studies by Saçan et al. (2007) and El-Sheekh et al. (2003) have shown various stimulatory effects of metals such as lead, aluminum, and cobalt at low concentrations in microalgae species. However, at levels surpassing the optimal level of metals and MNPs activate the inhibitory mechanisms in microalgae due to oxidative stress (Suman et al. 2015). The toxicity mechanisms of MNPs on microalgae are illustrated in Figure 1. The mechanism involves various pathways and the main mechanism is the formation of ROS due to the induction of oxidative stress by MNPs. MNPs induce oxidative stress in the cells by developing ROS. The ROS produced degrades and destroys the affected cell biomolecules such as proteins, and lipids as well as disrupts the biological activities as a response to the MNPs (Liang et al. 2020). The biochemical properties of *S. platensis* are affected when exposed to metals and MNPs due to the accumulation of reactive oxygen species (ROS), which results in the reduction of growth and photosynthetic rate as well as the degradation of proteins, phycobiliproteins, and carotenoids. Studies from El-Sheekh et al. (2003) and Zinicovscaia et al. (2017) have reported the degradation of biomass, and phenolic compounds, and decreased production of carbohydrates when microalgae were exposed to MNPs at high concentrations.

The formation of oxidative stress causes growth inhibition, reduction in proteins, lipids (Casazza et al. 2015), carbohydrates (Shilpi et al. 2014), chlorophyll (Deniz et al. 2011), carotenoids (Lone et al. 2013) and phenolic compounds (Comotto et al. 2014) of microalgae. The main components of *S. platensis* such as proteins, carbohydrates, and lipids are reported to be reduced significantly when exposed to selenium from 24 to 72 h (Zinicovscaia et al. 2017). Generally, metals and MNPs cause the inhibition of growth rate and also the production of biological macromolecules, pigments, and polyphenols. Table 2 shows the effect of metals and MNPs on *S. platensis*, while Table 3 reveals the effects of metals on *S. maxima.*

A study by Zinicovscaia et al. (2017) reported a reduction in *S. platensis* phycobiliproteins, especially phycocyanin (65.0%) and carbohydrates (76.0%) at 72 h after its exposure to selenium. Further, 80% reduction in biomass was found at 72 hrs due to the oxidation of the structural and functional components of *S. platensis*. Notably, *S. platensis* has a high efficiency to absorb metal ions from the environment through its various biochemical metabolic pathways especially due to its high ratio of surface area to volume (Shao et al. 2019). This is applicable in both viable and nonviable types of algae as the viable ones sequester metals compared to the non-viable ones where the carboxyl groups are

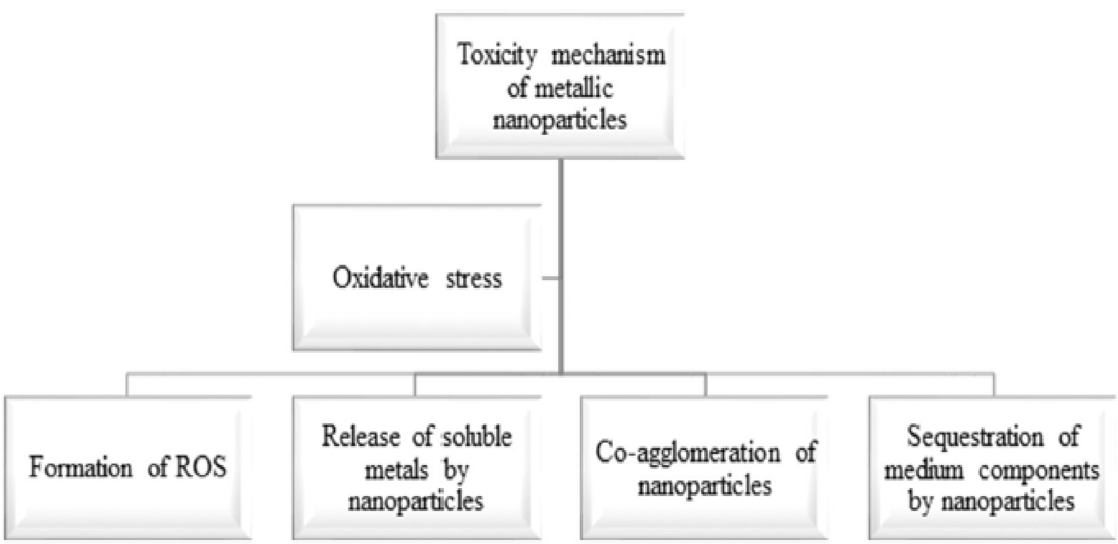

Figure 1 Toxicity mechanisms of Metallic Nanoparticles

Table 2 Effect of metals and metallic nanoparticles on *S. platensis*

Metals	Time (h)	Conc. (mg/L)	Effect	Reference(s)
Cr	216	10	62% reduction in biomass yield 64% reduction in chlorophyll-a 65% reduction in proteins 62% reduction in carbohydrates	(Shilpi et al. 2014)
Cu (II)	168	1	50% reduction in biomass yield 27% reduction in chlorophyll-a 20% reduction in carotenoids 13% increment in malondialdehyde	(Deniz et al. 2011)
Se	72	100	69% reduction in proteins 80% reduction in lipids 76% reduction in carbohydrates 90% reduction in phycocyanin	(Zinicovscaia et al. 2017)
TiO_2 NPs	504	100	74% reduction in biomass yield 24% reduction in phenolic compounds	(Comotto et al.2014)
TiO_2 NPs	120	500	66% reduction in lipids 12% reduction in polyphenols	(Casazza et al. 2015)
ZnO NPs	240	10	41% reduction in biomass yield 93% reduction in chlorophyll-a 50% reduction in carotenoids 79% reduction in proteins 78% reduction in nitrate reductase activity	(Lone et al. 2013)
ZnO NPs	96	200	76% reduction in biomass yield 76% reduction in carotenoids 74% reduction in phycocyanin	(Djearamane et al. 2018)

Table 3 Effect of metals on *S. maxima*

Metals	Time (h)	Conc. (mg/L)	Effect	Reference
Cd	504	1.2	30% reduction in biomass yield	(Augusto de Costa and de Franca 1998)
Cr(VI)	96	11.16	50% reduction in biomass yield	(Chen et al. 2003)
Zn	312	0.01mM	17% reduction in biomass yield	(Balaji et al. 2013)
Ni	312	0.01mM	33% reduction in biomass yield	(Balaji et al. 2013)

bound to the metal ions (Govindaraju et al. 2008). *S. platensis* sequesters metals in the aquatic environment while phytochelatin synthesis assists in the uptake of metals in microalgae (Rajamani et al. 2007). *S. platensis* binds to metals, especially polyvalent metals by its high binding capacity together with various functional groups assisting this function (Da Silva Vaz et al. 2016).

Zinc metal showed a 17% reduction in biomass yield after 312 hours of being exposed to *S. maxima.* Zinc oxide after 240 hours showed a report of a 79 % reduction in proteins of *S. platensis.* Most of the heavy metals such as chromium, copper, selenium, titanium oxide, etc., that were exposed to *S. platensis* caused a reduction in various parameters like biomass, chlorophyll-a, carbohydrates, carotenoids, phycocyanin and the phenolic compounds which ranged between 20% to 90%. Further, a study by Upasani et al. (2001) reported an increase in malondialdehyde upon treatment with copper indicating lipid peroxidation. An increase in lipid peroxidation is due to the accumulation of free radicals in the cell and destroys algal cells.

Conclusion and Future Recommendations

Algal cells have the potential to absorb metals and MNPs as the algae cell wall has the affinity in binding the metals, which explains the ability of *S. platensis* to absorb and retain metals and MNPs in the natural environment or bioremediation processes. The accumulation of certain metals in *S. platensis* is a potential risk for humans. Thus, proper evaluation of *S. platensis* on its contents is essential to avoid risks to human health. Metals that are found in the aquatic environment are usually more than one type, which could pose a synergistic effect causing a higher toxicity effect.

As metal contamination is becoming a global challenge, farmers are advised to check the quality of the water source used for the cultivation of *Spirulina*. The presence of metal pollutants does not only slow down the development of the *Spirulina*, the build-up of the metals in the cell can be a threat to human health. Although most of the studies found the metal pollutants contained in

Spirulina are still within the safety level for human consumption, the issue should be kept in check to limit the presence of the contaminants in the future.

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