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Challenges of using blooms of *Microcystis* spp. in animal feeds: a comprehensive review of nutritional, toxicological and microbial health evaluation

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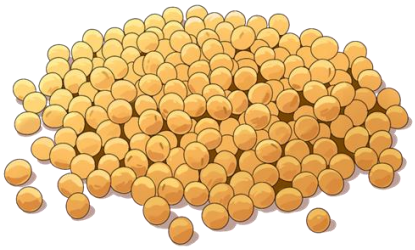
*Graphical Abstract



Soya bean: 37%-43% of crude protein (dry mass)

Microcystis spp.: 42%-60% of crude protein (dry mass)

Microcystis spp.: a substitutive source of protein in feed for animals?

***Highlights (for review : 3 to 5 bullet points (maximum 85 characters including spaces per bullet point)**

- ✓ Challenges of using blooms of *Microcystis* spp. in animal feeds were reviewed.
- ✓ *Microcystis* causes toxicity to mollusks, crustaceans, fish, amphibians, mammals and birds.
- ✓ *Microcystis* induces toxicity in liver, kidney, intestine, spleen and other organs.
- ✓ Fish fed *Microcystis* may be not safe for consumption for humans.
- ✓ Microbial pathogens may be present in cyanobacterial blooms.

Abstract

Microcystis spp., are Gram-negative, oxygenic, photosynthetic prokaryotes which use solar energy to convert carbon dioxide (CO_2) and minerals into organic compounds and biomass. Eutrophication, rising CO_2 concentrations and global warming are increasing *Microcystis* blooms globally. Due to its high availability and protein content, *Microcystis* biomass has been suggested as a protein source for animal feeds. This would reduce dependency on soybean and other agricultural crops and could make use of "waste" biomass when *Microcystis* scums and blooms are harvested. Besides proteins, *Microcystis* contain further nutrients including lipids, carbohydrates, vitamins and minerals. However, *Microcystis* produce cyanobacterial toxins, including microcystins (MCs) and other bioactive metabolites, which present health hazards. In this review, challenges of using *Microcystis* blooms in feeds are identified. First, nutritional and toxicological (nutri-toxicogical) data, including toxicity of *Microcystis* to molluscs, crustaceans, fish, amphibians, mammals and birds, is reviewed. Inclusion of *Microcystis* in diets caused greater mortality, lesser growth, cachexia, histopathological changes and oxidative stress in liver, kidney, gill, intestine and spleen of several fish species. Estimated daily intake (EDI) of MCs in muscle of fish fed *Microcystis* might exceed the provisional tolerable daily intake (TDI) for humans, 0.04 µg/kg body mass (bm)/day, as established by the World Health Organization (WHO), and is thus not safe. Muscle of fish fed *M. aeruginosa* is of low nutritional value and exhibits poor palatability/taste. *Microcystis* also causes hepatotoxicity, reproductive toxicity, cardiotoxicity, neurotoxicity and immunotoxicity to mollusks, crustaceans, amphibians, mammals and birds. Microbial pathogens can also occur in blooms of *Micr*ocystis. Thus, cyanotoxins/xenobiotics/pathogens in *Microcystis* biomass should be removed/degraded/inactivated sufficiently to assure safety for use of the biomass as a primary/main/supplemental ingredient in animal feed. As an ameliorative measure, antidotes/detoxicants can be used to avoid/reduce the toxic effects. Before using *Microcystis* in feed ingredient/supplements, further screening for health protection and cost control is required.

Key words: *Microcystis*, microcystin, feed, nutrition, toxicity, cyanotoxin, antidote, hazardous algal bloom, cyanobacteria, blue-green algae

Challenges of using blooms of *Microcystis* spp. in animal feeds: a comprehensive review of nutritional, toxicological and microbial health evaluation

1. Introduction

Cyanobacteria, commonly referred to as blue-green algae, are Gram-negative, oxygenic, photosynthetic prokaryotes which use energy from sunlight to convert carbon dioxide (CO_2) and nutrients into organic carbon products and biomass (Fogg et al., 1973; Whitton, 2012; Huisman et al., 2018). In recent decades, eutrophication, rising concentrations of CO_2 in the atmosphere and in surface waters, and global warming, have been associated with global increases in the frequency, intensity, duration and geographical spread of blooms of cyanobacteria in susceptible aquatic ecosystems. Cyanobacterial blooms can adversely affect water quality, including increased turbidity, hypoxia and anoxia, and the production of unpleasant taste and odor compounds (Deng et al., 2020). Of particular concern is the production of cyanobacterial toxins (cyanotoxins) by these organisms (van Apeldoorn et al., 2007; Metcalf and Codd, 2012; Chen et al., 2016a; Codd et al., 2017; Janssen, 2019; Svirčev et al., 2019).

To avoid or reduce risks associated with occurrence of blooms of cyanobacteria, many measures, including mechanical collection of biomass, physical adsorption and removal, use of chemical algaecides and biological control, have been-, and continue to be implemented in lakes (Chen et al., 2012, 2017b). Among these measures, in China, mechanical harvesting of cyanobacterial biomass is widely used as an urgent strategy during bloom seasons. After the drinking water crisis in Lake Tai (Ch: *Taihu*) in 2007 in China, over 1,000 tons of fresh cyanobacterial biomass is collected daily from May to August every year from Bays of Meiliang Gonghu, by the Wuxi Water Authorities. However, because water contents of harvested biomass are typically greater than 90%, dewatering, concentration, transportation, and further utilization of blooms are not cost-effective or energy-efficient (Chen et al., 2012).

Among freshwater cyanobacteria, *Microcystis* is one of the most commonly observed genera. Whilst *Microcystis aeruginosa* has been the most commonly reported and investigated species, a range of further morpho-species of *Microcystis* species are recognized, including *M. wesenbergii*, *M. ichthyoblabe*, *M. flos-aquae*, *M. viridis*, *M. botrys*, *M. panniformis*, *M. firma*, *M. natans*, *M. novacekii*, *M. smithii*, *M. bengalensis*, *M. ramosa*, and *M. Pseudofilamentosa* (Šejnohová and Maršálek, 2012; Harke et al., 2016; Bernard et al., 2017). Similar to other cyanobacteria, such as *Spirulina platensis* and some microalgae, including *Chlorella vulgaris*, *Microcystis*

spp. contains 42%-60% crude protein (dry mass basis), which is also similar to the protein content of soybean (37-43%) (Boyd, 1973; de la Fuente et al, 1977; de Moor and Scott, 1985; Qiao et al., 2013; Tables 1 and 2, Fig. 1). Therefore, *Microcystis* spp. bloom biomass have been suggested as a source for protein in animal feeds (Fig. 2). This could potentially reduce use of soybean and other agricultural crops and could make use of "waste" biomass when *Microcystis* scums and blooms are harvested (Fig. 3). *Microcystis* spp. contain further substances of potential nutritional value in addition to protein including lipids and poly-unsaturated fatty acids (PUFAs), carbohydrates, pigments, antioxidants, vitamins and minerals.

(Table 1)

(Table 2)

(Figure 1)

(Figure 2)

(Figure 3)

With the ever-increasing growth of human population and great demand for proteins and lipids, sustainable, alternative sources of these nutrients have become a priority due to asymptotic limitations of production of traditional crops under the burden of climate change and energy resource limits (Vanthoor-Koopmans et al., 2014; Colla et al., 2020). Thus, the potential for the use of non-toxic *Microcystis* blooms should be highlighted. However, several *Microcystis* species including *M. aeruginosa* are known to produce a diverse family of cyclic heptapeptides, microcystins (MCs) (Harke et al., 2016; Bernard et al., 2017; Svirčev et al., 2019), and MC- and non-MC-producing species/strains of *Microcystis* can occur simultaneously throughout blooms (Rinta-Kanto et al., 2005; Ha et al., 2009; Baxa et al., 2010; Wood et al., 2011; Harke et al., 2016). The general structure of MCs is: cyclo(-D-Ala1-L-X2-D-erythro-β-methylAsp(iso-linkage)3-L-Z4-Adda5-D-Glu(iso-linkage)6-N-methyldehydro-Ala7) (Catherine et al., 2017). More than 279 congeners of MCs have been described (Spoof and Catherine, 2017; Bouaïcha et al., 2019; Du et al., 2019). The L-amino acid residues 2 (X) and 4 (Z) contribute to the major varieties of MCs, and also determine the suffix in the nomenclature of these toxins. For instance, MC-LR includes leucine (L) and arginine (R) at positions 2 and 4, respectively.

MC-LR is one of the most common and toxic congeners and is also the most widely studied of the MCs (Metcalf and Codd, 2012; Chen and Xie, 2016; Svirčev et al., 2017). MCs have been found to accumulate in liver, kidneys, brain, testes and ovaries of vertebrates, where they can result in adverse outcomes (Chen et al., 2016a; Buratti et al., 2017; McLellan and Manderville, 2017). Based on doses, routes and duration of exposure, toxic effects including hepatotoxicity, nephrotoxicity, neurotoxicity, cardiovascular disease, immunomodulation, endocrine disruption, reproductive and developmental toxicity, and death can occur concomitantly or sequentially in animals exposed to MCs (Metcalf and Codd, 2012; Adamovsky et al., 2015; Chen et al., 2013, 2014, 2016ab, 2017b, 2018; Chen and Xie, 2016; Hu et al., 2016; Buratti et al., 2017; Svirčev et al., 2017). In 1998, the World Health Organization (WHO) established a provisional guideline value of 1 μg MC-LR/L in drinking water to protect human health against potential life-time exposure (WHO, 1998; Chorus and Bartram, 1999). In 2010, the International Agency for Research on Cancer (IARC) classified MC-LR as a Group 2B carcinogen, which is "possibly carcinogenic to humans" (IARC, 2010). In addition to MCs, *Microcystis* spp. can also produce other secondary metabolites which cause undesirable tastes and odors (odorous metabolites) or are biochemically active (bioactive metabolites) including cyanotoxins and protease inhibitors (Smith et al., 2008; Metcalf and Codd, 2012). Therefore, unlike *C. vulgaris*, the presence of MCs and other bioactive products potentially restricts the use of harvested *Microcystis spp.* (Gantar and Svirčev, 2008).

In addition to the presence of MCs, blooms of cyanobacteria and their mucilaginous layers potentially contain microbial pathogens which, if not removed or killed, may limit their use as a source of protein in feed (Deen et al. 2019; Vadde et al., 2019). In this review, challenges of using blooms of *Microcystis* spp. in animal feeds are identified.

2. Chemical composition and nutritional values of *Microcystis*

For *Microcystis* spp. to be considered as a potential food/feed ingredient, one crucial factor is their composition and nutritional content (Torres-Tiji et al., 2020). Chemical compositions vary significantly among *Microcystis* species/strains, and even within the same strains, nutritional content can vary tremendously according to environmental and growth conditions, the stage of cyanobacterial life cycle, geographic location and season (Mišurcová et al., 2014). Important nutrients and

components to consider are protein, lipid and carbohydrate content, as well as vitamin, mineral, and pigment content, all of which can positively affect animal health.

2.1. Proteins and amino acids

Protein is an essential macronutrient for animals and is regarded as the limiting nutrient for animal growth. It also accounts for major costs in the production of animal feed stuffs (Kong and Adeola, 2014). The relatively great protein contents of microalgae and cyanobacteria, including *Microcystis* spp., are a principal reasons for interest in the use of these organisms as non-conventional sources of protein. Interest in the potential use of microalgae and cyanobacteria as sources of single cell protein (SCP) began as early as the 1950s (Gouveia et al., 2008; Christaki et al., 2011). In general, the crude protein contents of *Microcystis* spp. (for example, about 56% of dry mass) compare favorably with those of some agricultural crops, such as soybean, corn and wheat, as well as microalgae (Table 2, de la Fuente et al., 1977; Lum et al., 2013).

Nutritional qualities of protein are determined by contents, proportions and availability of their constituent amino acids (Becker, 2007). Based on the Food and Agriculture Organization (FAO) protein reference pattern, the protein score of *Microcystis sp*. was calculated to be 42 (Table 3, de la Fuente et al., 1977). The total proportion of sulfur-containing amino acids is the most significant limiting factor in evaluating the nutritional value of *Microcystis* protein, which is similar to that of other potential sources of SCP, such as microalgae, yeasts and molds. The next most limiting amino acid is lysine, with a protein score of 81, with 70% of the total lysine content being chemically available to support growth (de la Fuente et al., 1977).

(Table 3)

Protein quality is an important criterion used to evaluate the nutritional values of novel ingredients in feeds and this depends on the profile and compositions of the relative amounts of amino acids, their solubilities, susceptibility to chemical and enzymatic hydrolysis in the digestive system, and their physiological utilization after absorption in the gastrointestinal tract (Tibbetts et al., 2015). All of the above factors can be affected by the source of feed ingredients, processing treatments, interactions with other dietary components, presence of anti-nutritional and toxic factors and the

feeding habits of target animals. The most accurate method to evaluate quality of proteins is to perform *in vivo* biological assays and calculate parameters including protein efficiency ratio (PER), biological value (BV), digestibility coefficient (DC) and net protein utilization (NPU) (Becker, 2007; Villarruel-López et al., 2017). PER is expressed as gain of body mass per unit protein ingested by animals during short-term feeding experiments. BV is based on the nitrogen (N) retained for growth or maintenance. DC is the proportion of protein digested by animals, compared to that hydrolyzed by chromic oxide (Cr_2O_3), an indigestible marker. Net protein utilization (NPU = DC × BV) is a measure of both digestibility of the protein consumed in food and the biological value of the amino acids. When *Microcystis* sp. was fed to Wistar rats (10.4% of protein content) as the only protein source, little was consumed and mortality was greater than that of the controls, whether or not the diet was supplemented with 0.4% DL-methionine (de la Fuente et al., 1977). However, when *Microcystis* sp. was supplied as 25% of the total protein of a corn-*Microcystis* diet (2.05% of protein contributed by *Microcystis* sp.), the PER was significantly improved. These results suggested a possible use of *Microcystis* spp. as a protein supplement to corn, or cereal-based diets for mono-gastric animals.

Using *in vivo* assays with animals to estimate quality of protein in diets is time-consuming and costly, whereas *in vitro* methods, which simulate digestion of protein by including proteolytic enzymes, provide alternative approaches (Tibbetts et al., 2015). Although not fully definitive, *in vitro* assays are widely considered as effective tools to predict potential quality of protein for use in industry and as a nutrient. This is because *in vitro* assays are relatively cheap, they do not use live experimental animals and results are quickly obtained by use of small numbers of samples. They can also complement data on chemical composition and amino acid profiles. *In vitro* digestibility of protein from *Microcystis sp.* was 69.5% (de la Fuente et al., 1977), which is less than that of other cyanobacteria and microalgae (78-94%, Tibbetts et al., 2015). Therefore, efforts should be made to devise practical techniques for improving the digestibility of *Microcystis* spp. protein.

2.2. Lipids and fatty acids

Lipids are essential components in animals and are used as feed additives. Cyanobacteria and microalgae produce various lipids, including glycolipids,

phospholipids (polar lipids), glycerolipids, neutral storage lipids and free fatty acids (FFAs) (Villarruel-López et al., 2017). Contents of lipids of *M. aeruginosa* ranged from 1330% of dry mass (dm), according to different strains and growth conditions (Piorreck et al., 1984; Sharathchandra and Rajashekhar, 2011; Da Rós et al., 2012; Zuo et al., 2018; Table 2).

Among lipids, fatty acids can constitute major constituents of cyanobacterial and microalgal biomass and occur in various forms. They occur as: phospholipids which form a phosphate group at position sn-3; as glycerolipids based on addition of glycerol esterified at positions sn-1 and sn-2; and as non-polar glycerolipids, which can be esterified at 1 (monoacyl-glycerol), 2 (diacyl-glycerol), or 3 positions (triacyl-glycerol, TAG) (Villarruel-López et al., 2017). *M. aeruginosa* contains saturated fatty acids (SFAs), mono-unsaturated fatty acids (MUFAs) and poly-unsaturated fatty acids (PUFAs). Most of the fatty acids are saturated SFAs (49-81%). Hexadecanoic acids (palmitic acids, C16:0) account for the highest proportions (24%-76%) (Piorreck et al., 1984; Walsh et al., 1997; Sharathchandra and Rajashekhar, 2011; Da Rós et al., 2012). *Microcystis* spp. also have a relatively great content of PUFAs (6-44%), including α-linolenic acid (C18:3) (ALA) and γ-linolenic acid (C18:3) (GLA) (Piorreck et al., 1984; Krüger et al., 1995; Walsh et al., 1997; Sharathchandra and Rajashekhar, 2011; Da Rós et al., 2012).

2.3. Carbohydrates

Carbohydrates are the most important sources of energy for cells, tissues and organs. In cyanobacteria and microalgae, carbohydrates are present as mono-saccharides or polymers (Villarruel-López et al., 2017). The most abundant mono-saccharides include glucose, rhamnose, xylose and mannose, and the polymers vary in size (di-, oligo-, and polysaccharides) and composition. In contrast to higher plants, cyanobacteria and microalgae do not contain hemicellulose or lignin. Compositions of carbohydrates vary among strains and versus growth conditions and can range from about 5 to 60% of dm (Bickel et al., 2000; Jin et al., 2005; Zuo et al., 2018). For example, in *M. aeruginosa*, compared to normal conditions of nitrate supply (e.g. 17.6 mM $NaNO_3$), the contents of soluble carbohydrates, mainly monosaccharides and some oligosaccharides, were significantly greater under non-N conditions (0 mM $NaNO_3$) (Zuo et al., 2018). The contents of insoluble carbohydrates,

mainly structural or stored poly-saccharides, were greater under both low-N (8.8 mM $NaNO_3$) and non-N conditions (Zuo et al., 2018).

2.4. Pigments

One of the most obvious characteristics of cyanobacteria and microalgae is their pigmentation and color (Gouveia et al., 2008). In general, each genus or species has its own particular combination of pigments and characteristic color. In addition to chlorophylls, which are the primary photosynthetic pigments and impart green coloration, microalgae and cyanobacteria also contain various accessory or secondary pigments, including carotenoids (orange coloration), xanthophylls (yellowish shade) and phycobilins (red or blue coloration) (Villarruel-López et al., 2017). These natural pigments aid in absorption of sunlight, increase efficacy of utilization of energy from light and protect cyanobacteria and algae from the harmful effects of sunrays (Christaki et al., 2011). Moreover, their function as anti-oxidants in photosynthetic cells including those of higher plants, and the algae and cyanobacteria, exhibits interesting parallels with their protective roles as anti-oxidants in animals and humans (Gouveia et al., 2008).

2.4.1. Chlorophylls

The proportion of chlorophylls in *M. aeruginosa* is 0.21-0.95% of dm (Piorreck et al., 1984). Chlorophyll-a is the only form of chlorophyll in most cyanobacteria, including *Microcystis* spp. (Gouveia et al., 2008). In addition to their use as colorants for food and pharmaceuticals, chlorophyll derivatives have health-promoting activities (Gouveia et al., 2008). These compounds are used in medicine as anti-inflammatory-, wound healing-, and calcium (Ca) oxalate-controlling agents and for internal deodorization. Chlorophylls can also decrease the risk of colon cancer due to the prevention of toxic and hyper-proliferative effects of heme in dietary red meat (de Vogel et al., 2005; Balder et al., 2006).

2.4.2. Carotenoids

Carotenoids are lipophilic pigments derived from 5-C (carbon) isoprene units which are enzymatically polymerized to form highly conjugated 40-C structures (with

316 up to 15 conjugated double bonds) (Cardozo et al., 2007; Sathasivam et al., 2019).
317 One or both ends of the carbon skeleton can undergo cyclization to form ring
318 β-ionone end groups, which can be substituted at various positions by oxo, hydroxy or
319 epoxy groups, to form various xanthophylls. There are more than 600 carotenoids in
320 nature and about 50 have pro-vitamin A activity, including α-carotene, β-carotene and
321 β-cryptoxanthin (Gouveia et al., 2008). Carotenoids are contained in cyanobacteria
322 and microalgae at 0.1-0.7% of dm (Christaki et al., 2011; Polyak et al., 2013).
323 Animals and humans are incapable of synthesizing carotenoids and thus must obtain
324 these compounds from the diet (Cardozo et al., 2007). Results of epidemiological
325 studies have demonstrated that diets rich in carotenoids could decrease risks of several
326 diseases, especially those in which free radicals are involved in initiation, including
327 arteriosclerosis, cataracts, multiple sclerosis and cancer (Gouveia et al., 2008).
328
329 **2.4.3. Phycobiliproteins**
330 Phycobiliproteins are deep-colored, water-soluble accessory pigments, including
331 phycoerythrins (red), phycocyanins (blue) and allophycocyanin (light-blue) (Gouveia
332 et al., 2008). They are formed with proteins linked covalently to tetra-pyrrolic
333 chromophoric prosthetic groups, i.e. phycobilins, including phycourobilin (PUB,
334 yellow), phycoerythrobilin (PEB, red), phycocyanobilin (PCB, blue) and
335 phycobiliviolin (PXB, purple). These pigments are used as highly sensitive
336 fluorescence markers in clinical diagnosis, and as natural colorants in food products
337 and cosmetics. Phycobiliproteins also exhibit many pharmacological properties, such
338 as anti-oxidant, anti-inflammatory, neuro-protective and hepato-protective effects
339 (Gouveia et al., 2008).
340
341 **2.5. Vitamins**
342 Microalgae and cyanobacteria can also produce broad spectrum of vitamins,
343 including A, B1, B2, B3 (niacin and niacinamide), B5 (pantothenic acid), B6, B7 (H,
344 or biotin), B9 (M, or folic acid), B12, C and E (Gouveia et al., 2008). Nevertheless,
345 contents of vitamins in *Microcystis* spp. are unknown.
346
347 **2.6. Minerals**

Microalgae and cyanobacteria can also be a source of macrominerals, including sodium (Na), potassium (K), calcium (Ca) and magnesium (Mg) and other microminerals, such as iron (Fe), copper (Cu), zinc (Zn) and manganese (Mn) (Christaki et al., 2011). Nevertheless, the mineral content *Microcystis* spp. in a potential nutritional context is unknown.

3. Challenges of using blooms of *Microcystis* spp. in animal feeds

(Figure 4)

3.1. Different sources of *Microcystis* biomass

It should be recognized that the chemical composition, including the nutrient value and MC content of *Microcystis* spp. vary considerably based on species, strains, environmental and growth conditions, the stage of cyanobacterial life cycle, geographic location and season (Mišurcová et al., 2014). Within a species, the MC-synthesizing genes may not be always expressed, and both MC-producing and non-MC-producing strains can co-exist (Janssen, 2019). Therefore, methods and/or processes necessary to treat *Microcystis* biomass from different sources may be different. Also, it is necessary to evaluate the optimum ratios of addition of *Microcystis* biomass for animal consumption.

3.2. Harvesting and treatment of *Microcystis* biomass

Research is in progress on the harvesting of *M. flos-aquae* biomass by multi-stage filtration methods (Zang et al., 2020). This is primarily in relation to nutrient removal and bloom control in lakes. The harvesting and processing of *Microcystis* biomass with the aim of producing feedstuffs, have not been studied. However, the harvesting and processing may affect the chemical compositions of the final products (Mišurcová et al., 2014). The finding of different protein efficiency ratios (PER) for several cyanobacterial and algal species, versus different post-harvest treatments, has revealed that the important role of process control of the harvested biomass (Becker, 2007).

3.3. Taste and acceptance of *Microcystis*-containing diets by animals

Microcystis spp. can produce secondary metabolites which cause undesirable tastes and odors (odorous metabolites) (Smith et al., 2008). Palatability of *Microcystis* biomass for terrestrial animals is not known. However, a progressive decrease in cyanobacterial grazing rate by tilapia (*Oreochromis niloticus*) and of opercular beat rates occurred when the proportion of toxic *M. aeruginosa* PCC7820 in their food was increased (Keshavanath et al., 1994).

3.4. Digestibility of *Microcystis*

Poor digestibility of *Microcystis* spp. has been observed in several studies. The *in vitro* digestibility of protein from *Microcystis sp.* was 69.5% (de la Fuente et al., 1977), which is less than that of other cyanobacteria and microalgae (78-94%, Tibbetts et al., 2015). The mean assimilation efficiency of *Oreochromis mossambicus* fed harvested *M. aeruginosa* bloom material was 51% for total organic matters, 64% for proteins and 76% for phosphorus (P), respectively (de Moor and Scott, 1985; Table 6). Examination of feces of fishes by transmission electron microscopy (TEM) showed that most *Microcystis* cell walls had become permeable allowing cellular contents to be released. Further digestion caused cell wall degradation. However, up to 25% of the cells appeared to be intact even after passing through the gut. Weak assimilation of radiolabeled *Microcystis* by roach (*Rutilus rutilus*) was detectable, and assimilation rates decreased with increasing proportion of *Microcystis* in a mixture with *Aphanizomenon* (Kamjunke et al., 2002a). Therefore, efforts should be made to improve the digestibility of *Microcystis* spp. protein.

3.5. Nutritional values of *Microcystis*

Use of *Microcystis* biomass in animal nutrition has not yet been discussed in terms of the nutritional value of biomass in the daily diet. Depending on the unknown cyanobacterial biomass vitamin and mineral content, the intake of an uncontrolled amount of *Microcystis* biomass could lead to the detrimental effect of some biomass components due to hypervitaminosis or the accumulation of some minerals and other nutritional ingredients.

In the case of the use of *Microcystis* biomass, very specific analyses and precise

calculations would have to be performed with respect to all limiting and other significant elements, as a precaution against potential accumulation and overdose. In further calculations of the daily intake it is necessary to take into account how many other important elements would have been ingested via 100 grams of *Microcystis* biomass (proteins, vitamins, minerals, etc.) and how much more would be needed through the traditional diet.

3.6. Effects of *Microcystis* on animal growth

Due to the possibility of poor digestibility of *Microcystis* spp. and possible low/imbalanced nutritional values discussed above, animal growth may be retarded or inhibited (**Table 4**). Also, *Microcystis* spp. can produce protease inhibitors, including aeruginopeptins, cyanopeptolins, micropeptins, microviridins, aeruginosins and microcins, which can inhibit the digestive proteases, trypsin and/or chymotrypsin (Smith et al., 2008). This would result in incomplete digestion of proteins, decreased nutritional intake and reduced growth rates of target species (Smith et al., 2008). Animal growth may also be decreased as a response to the re-allocation of energy to detoxification of MCs and other cyanotoxins and/or repair processes following intoxication (Smith et al., 2008; Ziková et al., 2010).

(Table 4)

3.6.1. Mollusks

Bioenergetics analysis showed that *M. aeruginosa* (CCAP 1450/10 and CCAP 1450/06) resulted in lesser rates of clearance, filtration, ingestion, absorption, and production of feces by zebra mussels, but resulted in a greater rate of production of pseudo-feces and pseudo-diarrhea (Juhel et al., 2006b). Net energy balance (NEB), representing the scope for growth (SFG) of mussels feeding on *M. aeruginosa* CCAP 1450/10, was less than that of mussels ingesting non-toxic diets. These results reveal a sub-lethal, stressful effects of MCs (particularly MC-LF) on feeding behavior and energy balance of zebra mussels.

3.6.2. Crustaceans

Both *M. aeruginosa* strain IZANCYA2, which produces mostly MC-LR, and strain IZANCYA6, which does not produce MCs, caused a decrease in the growth of juvenile crayfish (Vasconcelos et al., 2001). Acute exposure of post-larvae of white shrimp (*Litopenaeus vannamei*) to *M. aeruginosa* resulted in increased mortalities, stress, lethargy, decreased feeding behavior, soft shells, empty gastrointestinal tract, and whitish stomach and hepatopancreas (Morales-Covarrubias et al., 2016). The moults disappeared and the specific growth rate decreased in freshwater crayfish (*Paranephrops planifrons*) fed with diets containing *Microcystis sp.* CYN06 for 27 days (Clearwater et al., 2014).

3.6.3. Fishes

Oral exposure to *M. aeruginosa* resulted in lesser body mass of *Oreochromis mossambicus* after 15 days, but after 21 days a slight gain in body mass was observed (de Moor and Scott, 1985; Table 7). 1+ year roach (*Rutilus rutilus*) fed with *Microcystis* showed lesser growth rate, liver glycogen and muscle protein (Kamjunke et al., 2002ab). Oral exposure to *M. aeruginosa* scum at a dosage of 50 µg/MCs/kg bm/day for 28 days inhibited growth of common carp (Li et al., 2004). Inclusion of natural bloom biomass of cyanobacteria (mainly *M. aeruginosa*) in fish diets, also resulted in greater mortality and feed rate (FR), as well as reduced growth and condition factor (CF) in gibel carp (*Carassius auratus gibelio*) (Zhao et al., 2006a; Table 8), hybrid tilapia (*Oreochromis niloticus* × *O. aureus*) (Dong et al., 2009), hybrid sturgeon (*Acipenser baeri* × *A. gueldenstaedtii*) (Dong et al., 2011), and yellow catfish (*Pelteobagrus fulvidraco*) (Dong et al., 2012). Greater feed rate (FR), but lesser feed conversion ratio (FCR) or feed conversion efficiency (FCE), specific growth rate (SGR) apparent digestibility coefficient for dry matter/protein/energy (ADCd/ADCp/ADCe), protein retention efficiency (PRE) and energy retention efficiency (ERE) were also observed. However, there were no significant changes in mortality, body mass or total length in Sacramento splittail (Acuña et al., 2012b), and threadfin shad (Acuña et al., 2012a). The lack of such responses in the latter two studies may have been due to the small ratio of added cyanobacteria in the diet and correspondingly small doses of MCs used (Acuña et al., 2012a,b). Surprisingly, the dietary intake of cyanobacteria promoted growth of Nile tilapia, and the authors inferred that this species may be more tolerant to MCs than other fish species (Zhao et al., 2006b). Increased specific growth rate (SGR) and decreased feed conversion ratio

(FCR) were also observed in Indian major carp (*Labeo rohita* Ham.) fed with *M. aeruginosa* bloom material (Kütz) for 90 days (Das et al., 2013). In another study, female Nile tilapia were fed with diets containing *Microcystis* spp. biomass (5%, 20%), and *Arthrospira sp.* (20%) for 28 days (Ziková et al., 2010). For the last week, the fish exposed to diets containing 20% of *Microcystis spp.* showed a slightly reduced rate of growth, with the FCR ranking as: control < *Microcystis*-5% < *Arthrospira*-20% < *Microcystis*-20%. These results suggested that diet highly supplemented with *Microcystis* spp. might inhibit growth by excessive use of energy to enhance hepatic metabolism and detoxification of MC-LR and further metabolites (Ziková et al., 2010). Lesser doses of *M. aeruginosa* promoted the growth of goldfish (*Carassius auratus*), but greater doses inhibited growth (Liang et al., 2015).

Larvae of fishes were also affected by *M. aeruginosa* (Ghazali et al., 2010). A 12- day experiment was performed to compare the effects of two bloom samples of *M. aeruginosa* from Lalla Takerkoust reservoir with different profiles of MCs on the growth of larval common carp. Larvae were fed with diets containing *M. aeruginosa* (0.06 ng MCs/larva/day) for 12 days. Compared with controls, mass and standard length of larvae from the 9th day were lower. Decreased SGR was also observed. Moreover, accumulation of MCs by the larvae was negatively correlated with growth performance between two cyanobacterial treatments.

3.6.4. Amphibians

Body mass, body width measured as the spiracle and snout-vent length (SVL) of larval bullfrogs (*Rana catesbeiana*) fed with *M. incerta* for 8 days were significantly decreased compared to values of tadpoles at the start of feeding trials or those of controls maintained in growth medium containing no cyanobacteria or algae (Pryor, 2003). Similarly, body mass of *Xenopus laevis* fed with field *M. aeruginosa* for 21 days was smaller, compared with controls (Ziková et al., 2013). However, there was a significant increase of body mass of tadpoles of frogs (*Rana grylio*) fed either field *Microcystis sp.* for 3 days, or cultured *M. aeruginosa* NIES-90 for 7 days (Zhang et al., 2012). These inconsistent results could be due to different sensitivities of different species of amphibians.

3.7. Toxicity of *Microcystis* to animals

Unlike *Chlorella vulgaris*, MCs and other bioactive substances produced by *Microcystis spp.* and other cyanobacterial bloom-forming species, can cause toxicity to animals, including molluscs, crustaceans, fish, amphibians, mammals and birds.

3.7.1. Mollusks

(Table 5)

Accumulation of MCs in the muscles and significantly greater activities of GST in the gills, digestive glands and muscles were observed in *M. galloprovincialis* exposed to *M. aeruginosa* strain M6, which produces MC-FR, -LR and –WR, at 1.5 $\times 10^5$ cells/mL for 4 days even after depuration for 14 days (Fernandes et al., 2009).

Potential of 4 strains of *M. aeruginosa* to cause DNA damage in zebra mussels was studied after exposure for 7, 14, or 21 days (Juhel et al., 2007). Each of three strains (SAG 17.85, CCAP 1450/10 and CCAP 1450/06) produces unique profiles of MC-LF and MC-LR, whereas the 4th strain, SAG 48.80, does not produce MCs. Cell viability analysis showed that the doses of MCs to which the mussels were exposed did not cause cytotoxicy to the haemocytes from the posterior adductor muscle sinus. However, DNA damage in haemocytes, and the percentage of tail DNA (% tDNA) measured by the Comet assay, were increased in mussels fed the 3 MC-producing strains, but not in individuals fed with *M. aeruginosa* strain SAG 48.80. Moreover, the DNA damage appeared to be strain-dependent, and greater concentrations of MCs were associated with greater genotoxicity (Juhel et al., 2007).

Dietary exposure to *M. aeruginosa* also caused immunomodulatory effects in zebra mussels (Juhel et al., 2015). Fewer total hemocytes were observed after 14 days of exposure and thereafter, in mussels exposed to 3 MC-producing strains of *M. aeruginosa*, while there were no obvious changes of total hemocyte counts in mussels fed with non-MC-producingstrain SAG 48.80. The ratio of granulocytes to hyalinocytes was increased only on the 7th day in mussels fed the two most toxic strains, which suggested a potential short-term inflammatory response. Phagocytic rate and index were smaller in mussels fed *M. aeruginosa* containing greater concentrations of MCs. Lysozyme (LZM) activities in cell-free hemolymph (CFH) were greater in mussels exposed to MC-producing *M. aeruginosa* compared with MC-free controls on the 14th day but subsequently exhibited a time- and

543 dose-dependent increase.

544 Exposure to *Microcystis* spp. also causes toxicity to freshwater snails.
545 Accumulation of MCs, changes of activities of acid phosphatase (ACP), alkaline
546 phosphatase (ALP) and GST, and ultrastructural damage including to nuclei,
547 mitochondria, rough endoplasmic reticulum and lysosomes, were observed in the
548 hepatopancreas of the freshwater snail (*Bellamya aeruginosa*) exposed to *M.*
549 *aeruginosa* FACHB-905, which produces MCs at concentrations of 0.5×10^6 and 1
550 $\times10^6$ cells/mL for 15 days (Zhu et al., 2011). After a 15-day exposure to
551 MC-producing *M. aeruginosa* 905, the hepatopancreas of the freshwater pearl mussel
552 (*Hyriopsis cumingii*) contained the greatest MC-LR concentration, followed by gonad,
553 gill and muscle (Yang et al., 2012). Ninety-eight differentially expressed genes
554 involved in cytoskeletal assembly, transcription, signal transduction, cellular
555 metabolism and apoptosis were observed in the hepatopancreas. Following exposure
556 to MC-producing *M. ichthyoblabe* TAC95 for 8 days, the hepatopancreas of the
557 freshwater snail *Sinotaia histrica* showed microstructural changes including
558 vacuolization and separation of the basal lamina from cells (Xie et al., 2014). The
559 dose-dependent accumulation of MCs in soft tissue, increased activities of LZM, GST,
560 glutathione peroxidase (GPX) and superoxide dismutase (SOD) in haemolymph,
561 increased GSH, and suppressive effects on catalase (CAT) activities, were observed in
562 triangle sail mussels (*Hyriopsis cumingii*) fed MC-producing *M. aeruginosa*
563 FACHB-905 for 14 days (Hu et al., 2015). Exposure to *M. aeruginosa* FACHB-905
564 for 21 days also resulted in the accumulation of MCs in soft tissue and pathological
565 damage in the gills, digestive diverticula and stomach of triangle sail mussels (Wu et
566 al., 2017). 129 down-regulated and 147 up-regulated unigenes were identified in
567 whole soft bodies of the female freshwater snail (*Parafossarulus striatulus*) fed
568 cultured *M. aeruginosa* for 8 weeks, compared with those fed the green microalga,
569 *Scenedesmus obliquus* (Qiao et al., 2018). Dose-dependent increased activities of
570 amylase, cellulase and lipase were also observed in the digestive gland and stomach
571 of triangle sail mussels fed *M. aeruginosa* FACHB-905 for 1, 3, 7, and 14 days (Gu et
572 al., 2019). There were no significant changes of filtration rate, number of byssal
573 threads, whole body dry mass, total protein or glycogen content in marine mussel
574 *Mytilus galloprovincialis* fed *M. aeruginosa* LEGE 91094 for 14 days (Oliveira et al.,
575 2020). However, 4 up-regulated and 3 down-regulated proteins were observed in

digestive gland.

3.7.2. Crustaceans

(Table 6)

In freshwater crayfish (*Paranephrops planifrons*) fed with diets containing *Microcystis sp.* CYN06 for 27 days (Clearwater et al., 2014), MCs preferentially accumulated in the hepatopancreas and muscle, and this accumulation was greater with increased dietary concentrations. The uptake of MC-WR, a MC congener from the diet was the smallest, with a greater uptake of MC-AR, -LA, -LR, and greatest uptake of MC-RR. In contrast to the positive correlation between MC hydrophobicity and *in vivo* toxicity to the protozoan *Tetrahymena pyriformis* (Ward and Codd, 1999), the uptake of different variants of MCs by *P. planifrons* did not appear to berelated to their hydrophobicity.

Male grapsoid (burrowing) crabs (*Neohelice granulata*) were fed commercial rabbit feed for 7 weeks, supplemented with two strains of *M. aeruginosa*, NPDC1, which does not produce MCs, and NPJB, which produces mostly MC-LR (Sabatini et al., 2015). Concentrations of MC-LR in the hepatopancreas increased slightly during the first 3 weeks and subsequently began to decrease until 7 weeks. Contents of thiobarbituric acid-reactive substances (TBARS) were greater in exposed crabs than in controls during the first 3 weeks. GSH contents were less than in controls during the 6th and 7th weeks. Activities of SOD and GST were greater from the 3rd to 7th week in MC-exposed crabs compared to controls. These results suggested that accumulation of MCs and oxidative stress/injury are limited and reversed/recovered by a mechanism of depuration/detoxification based on conjugation with GSH, mediated by GST and activated activities of anti-oxidant and detoxifying enzymes, including SOD (Sabatini et al., 2015).

3.7.3. Fishes

(Table 7)

Oral exposure of common carp to *M. aeruginosa* scum (50 μg/MCs/kg, bm/day) for 28 days resulted in significantly greater activities of serum ALT and AST (Li et al., 2004). Ultrastructural alterations in hepatocytes were also observed, including swelling of the endomembrane system, including mitochondria, endoplasmic reticulum and Golgi body, vacuolization of cytoplasm and accumulation of lipid droplets. Diets containing *Microcystis* spp. induced significant changes of biomarkers for oxidative stress and detoxification in Nile tilapia (*O. niloticus*) (Jos et al., 2005; Puerto et al., 2011), and loach (*Misgurnus mizolepis*) (Li et al., 2005), as shown by increased lipid peroxidation (LPO) and altered activities and expressions of genes/proteins of antioxidant enzymes, including CAT, SOD, GR, GPX and GST. A significant decrease of Fulton's condition factor, loss of muscle mass (cachexia), micro- and ultra- structural changes in liver, kidney, gill, intestine, and ovary, greater activities of ACP, ALP, ALT and AST, lower activities of protein phosphatase 2A (PP2A), reduced protein contents, and a smaller ratio of RNA/DNA concentration in muscle, were also observed in Nile tilapia (Molina et al., 2005; Preeti et al., 2016; Abdel-Latif and Khashaba, 2017), Sacramento splittail (*Pogonichthys macrolepidotus*) (Acuña et al., 2012b), and threadfin shad (*Dorosoma petenense*) (Acuña et al., 2012a) exposed to *Microcystis* spp. via the diet. The inclusion of natural bloom biomass of cyanobacteria (mainly *M. aeruginosa*) in fish diets, also resulted in greater mortality, as well as reduced hepatosomatic index (HSI), hepatic ALT activities, and histopathological changes including vacuolization of hepatocytes in gibel carp (*Carassius auratus gibelio*) (Zhao et al., 2006a; Table 8), hybrid tilapia (*Oreochromis niloticus* × *O. aureus*) (Dong et al., 2009), hybrid sturgeon (*Acipenser baeri* × *A. gueldenstaedtii*) (Dong et al., 2011), and yellow catfish (*Pelteobagrus fulvidraco*) (Dong et al., 2012). Even after periods of depuration for 30 days, dose-dependent greater activities of LDH and SOD, greater concentrations of GSH and malondialdehyde (MDA), increased DNA fragmentation and DNA-protein crosslinks were observed in male Nile tilapia exposed to cultured *M. aeruginosa* for 7 days (Khairy et al., 2012).

Fish also exhibit various immune responses after oral intake of *M. aeruginosa*. Lesser doses of *M. aeruginosa* resulted in greater immunity of crucian carp

(*Carassius auratus*) and blunt snout bream (*Megalabrama amblycephala*), but greater doses were found to diminish immune function responses (Qiao et al., 2013; Xia et al., 2018). This discrepancy was attributed to hormetic responses (hormesis) to MCs (Qiao et al., 2013). Lesser doses of MC-producing *M. aeruginosa* promoted the growth of goldfish (*Carassius auratus*), but greater doses inhibited growth (Liang et al., 2015). In another study, altered concentrations of serum total protein, albumin, globulin, changes in albumin:globulin (A:G) ratio, and lysozyme activity, bactericidal activity, and blood superoxide anion production, were also observed in Indian major carp fed with field *M. aeruginosa* (Kütz) for 90 days (Das et al., 2013). However, compared to controls, higher survival percentages were observed after intraperitoneal infection of a lethal dose of *Aeromonas hydrophila*.

Phytoplanktivorous fishes, including silver carp, big-head carp (*Hypophthalmichthys nobilis*, previously: *Aristichys nobilis*) and tilapia (*Oreochromis niloticus*), are direct consumers of phytoplankton (including MC-producing *M. aeruginosa*) and zooplankton, and thus are widely used in the non-traditional bio-manipulation of cyanobacterial blooms (Xie and Liu, 2001; Lu et al., 2006; Zhang et al., 2008). In a laboratory study, the body mass of silver carp fed only *M.viridis* for 80 days did not significantly increase, but no mortality was observed (Xie et al., 2004). Silver carp and big-head carp exhibited rapid growth in fish pens located in Tai Lake (Ch:*Taihu*), where heavy cyanobacterial blooms (mainly *Microcystis* spp.) occurred during warm seasons (Ke et al., 2007, 2008; Guo et al., 2009, 2015; Zhou et al., 2009). The feeding intensities of these fishes were not decreased during blooms of *Microcystis* spp., but total gut content was the greatest. Since *Microcystis* species are usually less nutritious than zooplankton to big-head carp, the decreased growth in July and August may have been due to the increased percentage of *Microcystis* spp. (> 75%) in the guts (Ke et al., 2007).

Although the silver carp and big-head carp directly filter and feed on *Microcystis* spp. and therefore ingest MCs, which was shown by increased concentrations of MCs in intestinal contents and walls, these two fish species did not accumulate more MCs in liver and muscle and other tissues or organs, than other species, including herbivorous white amur bream (*Parabramis pekinensis*), grass carp (*Ctenopharyngodon idellus*), omnivorous crucian carp, common carp, muddy loach (*Misgurnus anguillicaudatus*), carnivorous redfin culter (*Chanodichthys erythropterus*,

previously: *Culter erythropterus*), top mouth culter (*Culter alburnus*, previously: *Culter ilishaeformis*), lake anchovy (*Coilia ectenes*), Taihu new silverfish (*Neosalanx taihuensis*) and yellow catfish (*Pseudobagrus fulvidraco*) (Xie et al., 2005; Chen et al., 2009ab; Zhang et al., 2009). During blooms of cyanobacteria, in comparison with carnivorous crucian carp and omnivorous top mouth culter, the phytoplanktivorous silver carp and bighead carp showed less liver damage (Li et al., 2007, 2008; Qiu et al., 2007). Biochemically, the phytoplanktivorous fish had greater basal GSH concentrations and showed higher correlations among the major anti-oxidant enzymes (CAT, SOD, GPX and GST) in liver, which may explain their increased resistance to MCs (Qiu et al., 2007). These results indicate that phytoplanktivorous fish may detoxify and degrade MCs more actively, and are probably more resistant to MC exposure, than other fish. These findings indicate potential to use these phytoplanktivorous species to help to tolerate and control toxic cyanobacterial blooms via non-traditional biomanipulation (Xie and Liu, 2001; Zhang et al., 2008).

3.7.4. Amphibians

(Table 8)

Concentrations of corticosteroids, including aldosterone and corticosterone, were smaller on the 3rd day but greater on the 21st day in *Xenopus laevis* fed with *M. aeruginosa* bloom material (Ziková et al., 2013). Surprisingly, no detectable accumulation of MC-LR or affected development was observed in tadpoles after exposure. Only minor, to negligible, up-regulation of transcriptions of luteinizing hormone (LH) in brain and heat-shock protein 70 (HSP 70) and multidrug resistance protein (MDR) in liver were found. These results suggested that *X. laevis* tadpoles have some mechanism(s) conveying resistance to cyanobacterial biomass containing MC-LR. However, serious histopathological damage was observed in the liver, intestine and skeletal muscle of tadpoles of *Lithobates catesbeianus* exposed to *M. aeruginosa* NPLJ4, which produces [D-Leu^{1}]MC-LR, for 16 days (Júnior et al., 2018). Samples showed signs of recovery after 15 days of depuration, but still with severe damage. No accumulation of free MCs in the tadpoles was observed by use of high performance liquid chromatography with photometric diode array detection

(HPLC-PDA) or mass spectrometry (MS) analysis.

3.7.5. Mammals

(Table 9)

The median lethal dose (lethal dose, 50%, LD_{50}) of *M. aeruginosa* cells for mice exposed to a sub-chronic, oral dose for over 21 days was 2.6 g dry *M. aeruginosa*/kg, bm (Falconer et al., 1994). Greater concentrations of plasma GGT, ALP, total bilirubin (BIL), lesser concentrations of plasma albumin, and microstructural changes in liver were found in pigs exposed to *M. aeruginosa* bloom material in drinking water for 44 days (Falconer et al., 1994; Chorus and Bartram, 1999). However, no significant changes of water or feed intakes, body mass gain, relative growth rates, plasma TBIL, GGT, ALP, GADH or AST activities, or liver mass were observed in lactating Holstein-Friesian cows or beef cattle which were orally exposed to *M. aeruginosa* (containing 0-13 µg MC-LR/kg bm/day) for 3-4 weeks (Orr et al., 2001, 2003; Feitz et al., 2002). While production of milk decreased during the experiment, the authors considered that this reduction might be due to stress, crush or the physiological response of mid-late lactating cows (Feitz et al., 2002). In the milk, less than 2 ng MC-LR/L (Orr et al., 2001) or 0.2 µg MC-LR/L (Feitz et al., 2002) was detected, nevertheless as the authors suggested, enzyme linked immunosorbent assay (ELISA) may generate false positive result and thus this finding required further investigation.

ELISA of the liver exposed beef cattle showed a concentration of 0.28-0.92 µg MC-LR/g, wm (Orr et al., 2003). However, no MCs were detected by HPLC or GC-MS. Based on results of ELISA, concentrations of MCs in livers of exposed individuals were > 1,000-fold greater than the limit of quantification (LOQ) by HPLC and GC-MS, which indicated that results of ELISA were due to cross-reaction with some chemicals other than MC-LR (Orr et al., 2003). This apparent anomaly may have been due to cross reactivity of MC antibodies with several naturally-occurring, metabolic MC-detoxification products (Metcalf et al., 2000, 2002). Daily intake of *Microcystis* for 28 days caused greater concentrations of serum cholesterol, creatinine, phosphorus, lipases, and smaller concentrations of ALT, bilirubin, cholinesterase in rats (Adamovsky et al., 2011). Significantly less red blood cell (RBC) counts and

mean corpuscular volumes (MCV), greater mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentrations (MCHC), greater hepato-, spleen-, and testis- somatic indexes, and microstructural changes in liver were also observed (Adamovsky et al., 2013; Palikova et al., 2013).

3.7.6. Birds

(Table 10)

Oral intake of *Microcystis* spp. resulted in histopathological damage of liver and testis, greater activities of plasma LDH, smaller concentrations of glucose, significant changes of antioxidant activities (GPX, GR, CAT), lipid peroxidation (LPO), and activities of activation phase (cytochrome P-450-dependent 7-ethoxyresorufin *O*-deethylase, EROD) and conjugation phase (GSH, GST) of detoxification in liver, brain, testis and heart of Japanese quail (*Coturnix coturnix japonica*) (Skocovska et al., 2007; Pašková et al., 2008; Damkova et al., 2009, 2011; Pikula et al., 2010; Paskova et al., 2011).

3.8. Accumulation of MCs in muscle and risk for human consumption

The results of several studies have shown that estimated daily intakes (EDI) of MCs via edible muscle of fish fed *Microcystis* spp. were close to or exceeding the tolerable daily intake (TDI) for the congener MC-LR of 0.04 µg/kg, bm per day for humans, established by the WHO and thus were not considered safe for human consumption (Li et al., 2004; Zhao et al., 2006b; Dong et al., 2009, 2011, 2012; Liang et al., 2015; Xia et al., 2018; Table 4). Even after periods of depuration of 43, 55 or 60 days, the ingestion of MCs would still have been greater than the WHO provisional guideline for health protection. It was therefore inferred that edible (muscle) tissue of fish exposed to *Microcystis* spp. under these conditions was not safe for human consumption (Dong et al., 2009, 2011, 2012). Results of some studies have indicated that the consumption of muscle of silver carp and big-head carp might be safe for humans (Chen et al., 2009b; Zhang et al., 2013), but others have indicated that these products are not always safe (Xie et al., 2005; Chen et al., 2006, 2007; Guo et al.,

772 2015), especially during blooms of *Microcystis* spp.

773

774 **3.9. Cyanotoxins and cyanobacterial metabolites beyond MCs**

775 *Microcystis* spp. which do not produce MCs can also be toxic to animals. For
776 example, diets containing the non-MC-producer *M. aeruginosa* IZANCYA6 caused a
777 decrease in growth of juvenile crayfish (Vasconcelos et al., 2001). Biomass of *M.*
778 *wesenbergii*, lacking detectable MC, also caused significant lethality to embryos of
779 the African clawed frog, and the estimated LC_{25} value was 232 mg biomass/L
780 (Dvořáková et al., 2002). *M. wesenbergii* also resulted in malformations, with EC_{50} of
781 about 300 mg biomass/L and EC_{25} of 75 mg biomass/L. Results showed that *M.*
782 *wesenbergii* biomass can present a significant risk of teratogenicity, and that the
783 teratogenic index (TI value) of 3.1 was even greater than that of *M. aeruginosa* (2.4).
784 Acute oral exposure of Patagonian pejerrey to *M. aeruginosa* NPDC1 which did not
785 produce MCs induced an increase in GST activity in liver (Bieczynski et al., 2013).
786 Decreased percentages of hyalinocytes and large basophilic hemocytes, and increased
787 lysozyme activitieswere observed in the hemolymph of zebra mussels after dietary
788 exposure to *M. aeruginosa* SAG 48.80, which did not produce MCs (Juhel et al.,
789 2015).

790 Certainly, MCs are not the only, mainly or necessarily the major toxic
791 components in *M. aeruginosa* (Falconer, 2007; Chen et al., 2016a). In addition to
792 MCs, *Microcystis* spp. can synthesize and release a wide range of other metabolites
793 including anatoxin-a, retinoic acids, microviridins, anabaenopeptins, aeruginosins,
794 microginins, piricyclamides and cyanopeptolins (Park et al., 1993; Park and Watanabe
795 1995; Osswald et al., 2009; Wu et al., 2012; Gemma et al., 2016; Otten et al., 2017).
796 Some strains of *Microcystis* spp. can produce at least two types of cyanotoxins (Park
797 et al., 1993). Furthermore, as Gram negative prokaryotes, cyanobacteria, including
798 *Microcystis spp.* characteristically contain lipopolysaccharide (LPS) endotoxin as
799 components of their cell walls (Stewart et al., 2006; Metcalf and Codd, 2012;
800 Monteiro et al., 2017). Also, multiple *Microcystis* species or strains can co-occur
801 within a population/community as a general condition (Metcalf and Codd, 2012;
802 Otten et al., 2017). Whilst the monitoring of MCs in *Microcystis* biomass, intended
803 for animal feed, is clearly a priority requirement, analysis for additional cyanotoxins
804 may also be necessary. Other toxins produced by *Microcystis* spp. and additional

cyanobacterial genera which might co-occur in biomass, may be additive or synergistic to the toxicity of MCs. Quantitative evaluation of the total toxicity induced by MCs and other secondary metabolites is a major scientific challenge and an important issue which needs to be addressed in future toxicological studies on *Microcystis* spp. and further cyanobacteria (Pavagadhi and Balasubramanian 2013; Chen et al., 2016a).

3.10. Additional toxic chemicals

Cyanobacteria can also bioaccumulate xenobiotics from aquatic environments, including heavy metals, polycyclic aromatic hydrocarbons (PAHs), organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs) and endocrine disrupting chemicals (EDCs) (Chen et al., 2017; Shi et al., 2017; Jia et al., 2018, 2019). As part of the selection process for the use of *Microcystis spp*. biomass as a potential source of protein (amino acids) in feed for animals, early testing to determine potential chemical contaminants is warranted (Fig. 5). These tests continue to be necessary after a decision to proceed is made and throughout biomass harvesting and processing. The need for analysis of xenobiotics is increased due to the ability of cyanobacteria to bioaccumulate these compounds in aquatic environments. Also, due to the potential for toxic potencies of as yet unidentified compounds and/or interactions among chemicals in mixtures, testing should not be based solely on instrumental monitoring of MCs and other toxicants, but also, should include bioassays, particularly including oral toxicity determination using mice or other animals. Finally, doses used in the bioassays with mice or other animals need to be scaled appropriately to allow proper interpretation and extrapolation of the results to fish, poultry, livestock, etc.

(Figure 5)

3.11. Different susceptibilities among species and optimum proportion of *Microcystis* biomass for feed

Examples of the different susceptibilities of different animal species to oral dosing with *Microcystis* biomass were given in Section 3.6. A single oral dose of *M. aeruginosa* 7806 (370 mg *M. aeruginosa* 7806/kg bm; 1,700 µg MC/kg bm) caused

mortality in common carp (*Cyprinus carpio*) within 24-48 hours, while the same dose resulted in no mortality of rainbow trout (Tencalla et al., 1994; Fischer and Dietrich, 2000). The phytoplanktivorous fishes, silver carp and bighead carp had greater basal GSH concentrations and higher correlations among the activities of major anti-oxidant enzymes (CAT, SOD, GPX and GST) in liver, enabling them to detoxify and degrade MCs more actively andprobably conferring more resistance to MC exposure than in other fish species (Li et al., 2007, 2008; Qiu et al., 2007). The absolute lethal dose (LD_{100}) of *M. aeruginosa* NRC-1 to mice, guinea pigs, and rabbits were all 3.2 g/kg, bm via the oral route (Konst et al., 1965). However, on an equivalent body mass basis, lethal doses for a lamb and two calves were 3-10 fold greater, i.e. 16 and 9.6-32 g/kg, bm, respectively. Symptoms were less pronounced and survival time was longer in the large ruminants than in laboratory animals. In chickens, oral doses required for lethality were 8-16 g/kg, bm (Konst et al., 1965).

In addition to this experimentally-determined variability, the established requirements for the identification and incorporation of environmental toxicology "safety factors" (Hughes, 1996; Chorus and Bartram, 1999) need to be included in decision-making on the suitability of *Microcystis* biomass for animal consumption. These factors include allowance for uncertainties regarding differences in susceptibility of animals to toxicants, both between- and within (e.g. versus sex and age) species. It is necessary to include these factors, typically 10-fold differences, in determining the health safety of material including cyanobacteria and the optimum ratios of addition for animal consumption.

3.12. Effects of *Microcystis* on quality of muscle

Goldfish and blunt snout bream fed with *M. aeruginosa* were of poor nutritional value and reduced quality of muscle (Liang et al., 2015; Wang et al., 2017). Significantly reduced concentrations of crude protein, crude fat and ash were observed in the muscle of goldfish which had consumed dietary *M. aeruginosa* (10%, 20%, 30%, 40% of cyanobacteria in diet) for 16 weeks (Liang et al., 2015). *M. aeruginosa* also resulted in greater concentrations of saturated fatty acids (SFA) in fish muscle, but lesser concentrations of n-3 poly-unsaturated fatty acids (PUFAs) and collagen, decreased pH and myofibril length, increased fiber diameters and cooking loss. Also, the concentrations of flavor compounds, including amino acids,

nucleotides, organic acids and carnosine exhibited significant changes in fish exposed to *Microcystis* and concentrations of compounds affecting taste and odor, including geosmin (GSM) and 2-methylisoborneol (MIB) were greater (Liang et al., 2015). Dietary *M. aeruginosa* (30% of cyanobacteria in diet) also caused lesser concentrations of total amino acids in dorsal muscle, total essential amino acids (EAAs), decreased gumminess, and increased pH of muscle of blunt snout bream (Wang et al., 2017). These results suggested decreased palatability, nutritional values and quality of muscle.

3.13. Microbial pathogens in *Microcystis* bloom material

In addition to the presence of MCs, blooms of cyanobacteria and their mucilaginous layers can contain microbial pathogens that, if not removed or killed, may limit use of biomass as a source of protein in feed. Blooms of cyanobacteria create a microenvironment that provides protection and a physical substrate, as well as nutrients and useful molecules, such as sugars and amino acids, which can be used as sources of carbon and energy for associated microbes. For instance, some bacteria benefit from and live in close association with heterocystous cyanobacteria capable of nitrogen fixation (Paerl, 1976). Also the general possibility of carbon-scavenging within blooms of cyanobacteria promotes growth of heterotrophic bacteria (Paerl, 1978).

Cyanobacterial blooms can contain pathogenic bacteria such as *Vibrio cholerae* (Islam et al. 1990, 1994; Epstein 1993). Potential environmental reservoirs of *V. cholerae* were recently reviewed by Islam et al. (2020). The authors evaluated several possible inter-epidemic reservoirs of *V. cholerae*, such as aquatic fauna, including zooplankton and crustaceans, and flora, including macrophytes, cyanobacteria and microalgae. Some species of cyanobacteria, including *Anabaena variabilis* and *M. aeruginosa*, are able to act as inter-epidemic reservoirs of *V. cholerae*. Screening of cyanobacterial (*Spirulina*) and microalgal biomass harvested from open systems for faecal coliforms was negative, but positive for faecal streptococci (Jaquet, 1976), which is an early indication of the need for microbial assessment of such biomass (Jassby, 1988). Much of the work on the association between *V. cholerae* and cyanobacteria has concentrated on surface waters in Bangladesh, but there is no reason that such associations are unique to conditions in this location. Besides

Bangladesh, cholera is endemic in parts of India and outbreaks of the disease have been reported in Yemen, as well as in regions of sub-Saharan Africa and Haiti (Deen et al. 2019). Also, due to the cultural eutrophication of limited freshwater resources and high water temperatures, cyanobacteria can thrive in many of these countries. While China is not among the countries hardest hit by cholera (Deen et al. 2019), many faecal-associated and pathogenic bacteria occur in the Tiaoxi River, a major inflow entering Lake Taihu in China, where they can become associated with *Microcystis* blooms (Vadde et al., 2019).

There is thus abundant evidence that pathogenic microorganisms including bacteria, fungi and virus can be present in *Microcystis* blooms. Thus, before and/or after processing, bloom biomass intended for use as feed for animals, must be analyzed for microbial pathogens as part of the decision-making process.

4. Measures to avoid or ameliorate toxic effects caused by *Microcystis*

(Figure 6)

Due to reports of poisonings/intoxications of animals and humans and evidence that *Microcystis* spp. and MCs cause or contribute to these events, MCs have received increasing attention, particularly as a threat to public health (Metcalf and Codd, 2012; Chen et al., 2016a; Buratti et al., 2017; Svirčev et al., 2017, 2019). Cyanotoxins and xenobiotics should be removed or degraded sufficiently to assure that the threshold for toxicity of the *Microcystis* biomass is not exceeded in products intended for use as a primary (main) or supplemental protein source in animal feed. As an ameliorative measure, antidotes or detoxicants can be used to avoid or reduce the toxic effects caused by MCs and additional cyanotoxins. Here, some trials to ameliorate toxic effects caused by *M. aeruginosa* are reviewed.

4.1. Removal of MCs and other bioactive metabolites produced by *Microcystis* spp. and xenobiotics accumulated by *Microcystis* spp.

Recently, the acidolysis product of materials from blooms of *M. aeruginosa* in Lake Tai by hydrochloric acid (HCl) treatment and heating was reported to be safe for use as an ingredient of animal feed (Han et al., 2015). After extraction of *M.*

aeruginosa by heating and acidolysis using HCl, the concentrations of amino acids, residual MCs, and heavy metals of the acidolysis product were determined. After 18 hours of heating and acidification, the product contained 17 identified amino acids. These amino acids accounted for 51% of total acidolysis product, and 30% of the remainder were essential amino acids (EAAs) for livestock and poultry. The concentration of residual MC-LR was 0.94 μg/kg, dm, which was less than the WHO provisional guideline value of MCs for human drinking water limit (1 μg MC-LR/L, equal to 1 μg MC-LR/kg). Concentrations of As, Pb, Hg and Cr in the feed were in compliance with National Standards of China for feed (Han et al., 2015). Furthermore, the results of Horn's method showed the LD_{50} dose of the acidolysis product to ICR mice via the oral route was greater than 9.09 g/kg, bm, which was classified as being non-toxic (5,001-15,000 mg/kg) (Han et al., 2015). None of the oral dosages of 2.15, 4.64, 10 or 21.5 g/kg, bm of the *M. aeruginosa* acidolysis product resulted in significantly greater activities of hepatic ALP, serum LDH or γ-glutamyltransferase (γ-GT). The acidolysis product exhibited neither mutagenicity nor effects on sperm malformation. It was concluded that the product was safe for use as an animal feed ingredient (Han et al., 2015).

Water treatment technologies to remove toxic compounds may be used for the treatment of *Microcystis* spp. biomass, including disinfection processes and the destruction of cyanotoxins by common oxidants, including free chlorine (NaOCl), chlorine dioxide (ClO_2), chloramines (mainly monochloramine, NH_2Cl), permanganate ($KMnO_4$) and ferrate (FeO_4^{2-}), adsorption by activated carbon, iron-based adsorbents, and advanced oxidation processes including ultrasound, ultraviolet (UV), UV/H_2O_2, and ozone (He et al., 2016; Zhou et al., 2019). However, unlike in water treatment technologies, the cyanobacterial proteins and other nutrients intended for animal nutrition should not be affected.

Properly processed products containing *Microcystis* spp. might be safe for use as an ingredient in animal feed if the following two requirements are met:

1) Cyanotoxins and other bioactive metabolites produced by *Microcystis* spp., including MCs, and xenobiotics accumulated by blooms of *Microcystis* spp., e.g. heavy metals and pesticides, are sufficiently removed to prevent significant oral exposure to cause adverse health outcomes in the animals being fed and in subsequent human consumers of products of those animals;

2) No toxicants are produced or introduced during the harvesting and processing of the *Microcystis* spp. biomass.

4.2. Potential use of antidotes to ameliorate toxic effects caused by *Microcystis*

Microcystis or MC-induced toxicity in fish and mammals can be ameliorated to different degrees by antidotes or detoxicants, including: 1) transporter inhibitors, e.g. bile acids, cyclosporin A, rifampicin, cytochalasin, trypan blue, trypan red and naringin; 2) anti-inflammatory agents, e.g. several glucocorticoids, including fluocinolone, dexamethasone, and hydrocortisone; 3) osmotic agents, e.g. D-glucose, mannitol, and dihydroxy acetone; and 4) antioxidants, e.g. GSH, N-acetylcysteine, L-cysteine, vitamin C, vitamin E, selenium, melatonin, flavonoids, quercetin, silybin, and morin, and green tea polyphenols, primarily catechins, and sulforaphane (Guzmán-Guillén et al., 2017). Some results concerning the elimination or alleviation of MC effects by antidotes have been summarized by Guzmán-Guillén et al. (2017). Trolox, a soluble vitamin E analogue (Prieto et al., 2008, 2009; Table 11), sodium selenite (Na_2SeO_3) pentahydrate (Atencio et al., 2009), and N-acetylcysteine (NAC), a precursor of glutathione (GSH) (Puerto et al., 2009, 2010), can ameliorate histopathological damage and oxidative stress caused by *Microcystis* spp. in fish. Naringin can inhibit the uptake of MC-LR in the freshwater snail exposed to *M. ichthyoblabe* (Xie et al., 2014).

(Table 11)

4.2.1. Naringin

Naringin, a flavonoid isolated from grape and citrus fruit species, exhibits therapeutic potential, including anti-oxidant, anti-inflammatory, anti-ulcer, anti-atherogenic, anti-cancer, hepato-protective and neuro-protective activities (Xie et al., 2014). It is also a clinical inhibitor of the organic anion-transporting polypeptide 1A2 (OATP1A2).

Naringin was shown to inhibit accumulation of MC-LR by freshwater snails (Xie et al., 2014). Initial treatment with 1 mM (0.581 g/L) naringin only on the first day resulted in prevention of accumulation of MC-LR in the hepatopancreas of the freshwater snail *S. histrica* exposed to *M. ichthyoblabe* TAC95 by approximately 60%

over a subsequent 8 days. Initial treatment with 10 mM naringin only on the first day suppressed MCs accumulation in the first 2 days, but concentrations of MC-LR increased in the animals from the 5th to 8th day. With continuous treatment of 10 mM of naringin for 8 days, the MCs uptake was completely prevented.

4.2.2. Vitamin E

Vitamin E is a generic descriptor for all compounds which have the biopotency of α-tocopherol (Prieto et al., 2008). All of the natural forms of vitamin E are D-stereoisomers and consist of a substituted aromatic ring and a long isoprenoid side chain. Among the 8 natural molecules with vitamin E activity, α-tocopherol exhibits the greatest biological activities. Vitamin E is a lipid-soluble anti-oxidant which can decrease LPO and thus can protect biological membranes against free radical-induced damage.

Effects of pre-treatment with Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid), a water-soluble vitamin E analogue, on MC-induced oxidative stress/damage in the liver, kidney, and gill of male Nile tilapia were investigated (Prieto et al., 2008; Table 8). The fish were fed Trolox (at about 1.2, and 4.2 mg vitamin E/kg, bm/day) for 7 days and then were fed a single oral dosage of *M. aeruginosa* (about 2,400 μg MC-LR/kg, bm) in the diet. *M. aeruginosa* caused greater LPO values in liver, while the fish pre-treated with vitamin E showed no alteration. Activities of anti-oxidant enzymes, including CAT, SOD, GPX and GR, were also ameliorated by vitamin E-dosing, while protein oxidation and the GSH/GSSG ratio did not exhibit obvious changes. The larger dosage of vitamin E (about 4.2 mg vitamin E/kg, bm/day) had a greater protective efficacy, especially upon LPO and CAT activity (Prieto et al., 2008).

The time-dependent protective actions of Trolox on oxidative stress/injury and histopathological damage caused by MC-producing *M. aeruginosa*, were also studied (Prieto et al., 2009). Male Nile tilapia were fed Trolox supplement (about 4.2 mg vitamin E/kg, bm/day) for 7 days and then fed a single oral dose of *M. aeruginosa* (about 2,400 μg MC-LR/kg, bm) in the diet, and sacrificed after 24, 48, or 72 hours. For the biomarkers of oxidative stress including LPO, SOD, CAT, GR and GST in liver, kidney and gill changed by *M. aeruginosa*, a greater protective efficacy of vitamin E was observed 24 hours post exposure to *M. aeruginosa*, although protection extended for up to 48 hours in gills for some biomarkers. Treatment with Trolox also

reduced micro- and ultra-structural injury in liver, kidneys, gastrointestinal tract and heart, and the protective abilities were more obvious after 72 hours (Prieto et al., 2009).

4.2.3. Selenium (Se)

Selenium (Se) plays dual but contradictory roles in animals because it is both a nutrient and a toxicant (Atencio et al., 2009). Se can protect cells against oxidative stress as an integral component of GPX which contains 4 sub-units and every sub-unit includes a Se atom. Se is also a component of iodothyronine deiodinase and thioredoxin reductase, which is the only enzyme found to catalyze the reduction of thioredoxin and is involved in modulation of cellular redox homeostasis and protection against oxidative stress/injury. However, Se also can be toxic by reaction with sulfhydryl groups to generate biologically active reactive oxygen species (ROS). As a nutrient, the requirement of dietary Se for fish is 0.1-0.5 μg/g dm. However, Se becomes toxic at only 7-30 times the required dietary concentrations for nutrition (>3 μg/g, dm). Therefore, it is critical for homeostatic regulation that optimal amounts of Se are available to protect cells/tissues/organs from ROS-induced oxidative stress and to maintain overall health.

The protective effects of dietary Se supplement (sodium selenite, Na_2SeO_3) against morphological alterations and oxidative stress/damage caused by MC-containing *Microcystis sp.* in male Nile tilapia were investigated (Atencio et al., 2009). Fish were fed $NaSeO_3$ (about 10, 18, and 36 μg Se/kg, bm/day) for 7 days and then fed a single oral dose of *Microcystis sp.* (about 2,400 μg MC-LR/kg, bm), and killed after 24 hours. The protective actions of Se were dose-dependent. The lesser dosage of Se caused CAT and GR activities in liver and CAT and SOD activities kidney to converge to baseline values, but the reversal of LPO values and SOD and GST activities in liver required the greater dosage. However, Se induced a pro-oxidant effect with increased LPO in kidney and GPX activities in liver and kidney of fish which were not exposed to *Microcystis* sp. Amelioration of *Microcystis* sp.-caused pathological changes in liver, gastrointestinal tract, kidney and heart was observed by the greatest dosage of Se. Therefore, careful attention should be paid to control the amounts of Se supplementation to promote beneficial effects and to avoid potential detrimental outcomes.

4.2.4. N-acetylcysteine

N-acetylcysteine (NAC) is a derivative of the natural amino acid L-cysteine (L-Cys). It is a thiolic anti-oxidant, and a mucolytic agent for various respiratory diseases, and can act against cellular degeneration (Puerto et al., 2009). It is a precursor as a supplier of cysteine (Cys) in the synthesis of GSH and can stimulate activities of cytosolic enzymes involved in the GSH cycle, including GR, enhancing the rate of GSH re-generation from GSSG. NAC can also protect cells by reacting with ROS.

The protective role of NAC against the oxidative stress/damage (Puerto et al., 2009) and pathological changes (Puerto et al., 2010) induced by MC-containing *Microcystis* sp. in male Nile tilapia were investigated. Tilapia were fed NAC (about 400, 880 and 1,936 mg/kg, bm/day) for 7 days and then fed a single oral dose of *Microcystis sp.* (about 2,400 µg MC-LR/kg, bm), and killed after 24 hours. NAC exerted a dose-dependent protective role (Puerto et al., 2009). Smaller doses of NAC ameliorated the increased LPO in liver and kidney and the decreased protein content and GSH/GSSG in liver caused by *Microcystis* sp.. Greater activities of anti-oxidant enzymes, including SOD, GPx, and GR, induced by *Microcystis* sp., were also recovered by NAC pretreatment. However, the greatest dose of NAC caused changes of some enzyme activities. Similarly, results of histopathology in the liver, kidneys, gills, intestine and heart suggested protective actions of NAC mainly at the median dose (880 mg NAC/kg, bm/day) probably due to its anti-oxidant activity (Puerto et al., 2010). However, the greatest dose of NAC caused toxic effects in fish not exposed to *Microcystis* sp. Thus, NAC can be used as a chemo-protectant in the prophylaxis, prevention and treatment of MC-induced toxicity induced by *Microcystis sp.* in fish. However, the optimal dose of NAC must be selected carefully because of its own pro-oxidant and pathological activities, which were induced by 1,936 mg NAC/kg, bm/day in these studies (Puerto et al., 2009, 2010).

Finally, it is emphasized that the concentrations of antidotes applied to ameliorate toxic effects caused by *Microcystis* spp. in dosed animals should be well controlled to avoid possible risks to health of humans, or other consumers of livestock, poultry or fish, which were fed diets containing *Microcystis* spp.

5. Cost control

Cyanobacteria such as *Spirulina platensis* and microalgae including *Chlorella vulgaris*, are expensive to culture and produce, although efforts are under way addressing cost-efficient mass cultivation of these organisms (Vanthoor-Koopmans et al., 2014). Compared with cyanobacteria such as *Spirulina platensis* and microalgae including *Chlorella vulgaris*, *Microcystis* biomass is highly available from naturally occurring scums and blooms, and it is not necessary to culture *Microcystis*, so there are no costs for production of *Microcystis* biomass. However, cost control for harvesting and treatment of *Microcystis* biomass, including dewatering, drying and feed production is needed. Also, costs for measures to avoid/ameliorate effects of toxic *Microcystis* biomass, including removal of MCs, other bioactive metabolites and xenobiotics accumulated by *Microcystis*, and use of antidotes, are needed to be considered. Pragmatic large-scale techniques must be devised to reduce the costs of treament of *Microcystis* biomass and to compete with conventional protein sources.

6. Conclusions

*Micr*ocystis spp., including *M. aeruginosa,* are rich in protein and have been suggested as a source of protein in animal feeds. However, in addition to protein, *Microcystis* spp. need to be considered as sources of a wider range of nutrients and metabolites including nutritionally beneficial components but also animal and human health hazards. They can produce unpleasant taste and odor compounds, and potent toxins including MCs, which can cause multiple toxicities to animals and humans, including hepatotoxicity, reproductive toxicity, developmental toxicity, nephrotoxicity, neurotoxicity, immunomodulation, endocrine disruption, and death. Without effective procedures to monitor and, if necessary, to remove or detoxify cyanotoxins, the presence of these potent toxins restricts the use of *M. aeruginosa* as a source of protein in animal feedstuffs. Here results of studies of nutritional and toxicological aspects of of *Microcystis* spp. on fish, mollusks, crustaceans, amphibians, mammals and birds have been reviewed. Inclusion of *M. aeruginosa* in diets has resulted in increased mortality, decreased growth, and caused toxicities, such as hepatotoxicity, gastrointestinal toxicity, nephrotoxicity, cardiotoxicity, neurotoxicity, immunotoxicity and animal deaths. The estimated daily intake (EDI) values of MCs in muscle of fish fed with *Microcystis* spp. are close to or exceed the tolerable daily intake (TDI)

derivation of 0.04 μg/kg, bm/day for humans, established by the WHO, and are thus not safe for consumption by humans. In addition, microbial pathogens can be present in *Micr*ocystis blooms. Properly processed products containing, or originating from *Microcystis spp.* might be safe for use as an ingredient in animal feed if the following three requirements are met: 1) toxicants produced by *Microcystis* spp., including MCs, additional cyanotoxins, and xenobiotics accumulated by *Microcystis* spp., including heavy metals and pesticides, and microbial pathogens are sufficiently removed or inactivated, to prevent significant exposure to cause adverse health outcomes in the animals being fed, or in consumers of products of those animals; 2) no toxicants or microbial pathogens are produced or introduced during processing of *Microcystis* spp.; 3) taste and odor compounds produced by *Microcystis* spp. should be removed. Antidotes or detoxicants can be used to avoid or reduce toxic effects caused by MCs and *Microcystis* spp. Before the use of *Microcystis* spp. as sources of protein for animal feed, further, well-designed and relevant investigations and safety and health evaluation are required. Also, cost control for harvesting and treatment of *Microcystis* biomass, feed production and measures to avoid/ameliorate toxic effects is needed to be considered.

Author contributions

Liang Chen conceived the idea and wrote the first draft manuscript.. John P. Giesy, Ondrej Adamovsky, Zorica Svirčev, Jussi Meriluoto, Geoffrey A. Codd and Biljana Mijovic wrote parts of the manuscript. Liang Chen, Ting Shi, Ondrej Adamovsky and Geoffrey A. Codd drew the figures. Liang Chen, John P. Giesy, Geoffrey A. Codd, Xun Tuo, Shang-Chun Li, Jun Chen and Ping Xie revised then edited the manuscript. All co-authors contributed to and checked the final draft.

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Conflicts of interest

None.

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Table 1. Chemical composition and nutritional values of *Microcystis* sp. (de la Fuente et al., 1977).

Component	% of *Microcystis* sp. (dry mass)
Moisture	10.4
Protein (N × 6.25)	55.6
Ether extract	1.2
Crude fiber	1.6
Ash	5.2
Nitrogen-free extract	26.0
Ribonucleic acid	7.4
Deoxyribonucleic acid	0.3
Caloric value (kcal)	540.3
Calcium (mg)	1,169.1
Phosphorus (mg)	633.4

Table 2. General nutritional composition of conventional feed ingredients, microalgae and cyanobacteria including *Microcystis* spp. (% of dry mass).

Source	Protein	Carbohydrates	Lipids	References
Soybean	37	30	20	Lum et al., 2013
Corn	10	85	4	Lum et al., 2013
Wheat	14	84	2	Lum et al., 2013
Anabaena cylindrica	43-56	25-30	4-7	Lum et al., 2013
Arthrospira maxima	60-71	13-16	6-7	Lum et al., 2013
Chlorella vulgaris	51-58	12-17	14-22	Lum et al., 2013
Spirogyra sp.	6-20	33-64	11-21	Lum et al., 2013
Synechococcus sp.	73	15	11	Lum et al., 2013
Microcystis aeruginosa	42.14	NA	NA	Boyd, 1973
Microcystis sp.	55.6	NA	NA	de la Fuente et al, 1977
M. aeruginosa	23.5-50.1	NA	13.7-23.4	Piorreck et al., 1984
M. aeruginosa	51-60	NA	NA	de Moor and Scott, 1985
M. aeruginosa NIVA-CYA 228/l	35-72	7-57	NA	Bickel et al., 2000
M. aeruginosa FACHB 41	NA	26-52	NA	Jin et al., 2005
M. aeruginosa	NA	NA	18.48-30.32	Sharathchandra and Rajashekhar, 2011
M. aeruginosa NPCD-1	NA	NA	13.19-28.10	Da Rós et al., 2012
M. aeruginosa 3123	51.3-69.5	NA	NA	Servaites et al., 2012
M. aeruginosa FACHB 469	22-55	NA	NA	Li et al., 2014
M. aeruginosa FACHB-912	NA	38.9-60.5	16-18	Zuo et al., 2018

NA: not analysed.

Table 3. Composition of amino acids of conventional protein sources, microalgae and cyanobacteria including *Microcystis* spp., and the Food and Agriculture Organization (FAO) protein reference pattern (g/100 g protein) (Boyd, 1973; de la Fuente et al., 1977; Becker, 2007)

	WHO/FAO	Egg	Soybean	*Chlorella vulgaris*	*Dunaliella bardawil*	*Scenedesmus obliquus*	*Arthrospira maxima*	*Spirulina platensis*	*Aphanizomenon* sp.	*Microcystis aeruginosa* (Boyd, 1973)	*Microcystis* sp. (de la Fuente et al., 1977)
Ile	4	6.6	5.3	3.8	4.2	3.6	6	6.7	2.9	5.57	7.55
Leu	7	8.8	7.7	8.8	11	7.3	8	9.8	5.2	8.75	8.88
Val	5	7.2	5.3	5.5	5.8	6	6.5	7.1	3.2	6.20	7.40
Lys	5.5	5.3	6.4	8.4	7	5.6	4.6	4.8	3.5	5.23	5.41
Phe	6a	5.8	5	5	5.8	4.8	4.9	5.3	2.5	4.23	4.19
Tyr		4.2	3.7	3.4	3.7	3.2	3.9	5.3	-	4.00	3.22
Met	3.5b	3.2	1.3	2.2	2.3	1.5	1.4	2.5	0.7	1.73	0.90
Cys		2.3	1.9	1.4	1.2	0.6	0.4	0.9	0.2	0.28	0.92
Try	1	1.7	1.4	2.1	0.7	0.3	1.4	0.3	0.7	-	1.51
Thr		5	4	4.8	5.4	5.1	4.6	6.2	3.3	5.68	4.31
Ala		-	5	7.9	7.3	9	6.8	9.5	4.7	7.88	9.13
Arg		6.2	7.4	6.4	7.3	7.1	6.5	7.3	3.8	9.71	10.06
Asp		11	1.3	9	10.4	8.4	8.6	11.8	4.7	12.95	14.93
Glu		12.6	19	11.6	12.7	10.7	12.6	10.3	7.8	12.81	13.74
Gly		4.2	4.5	5.8	5.5	7.1	4.8	5.7	2.9	4.79	5.25
His		2.4	2.6	2	1.8	2.1	1.8	2.2	0.9	1.40	1.49
Pro		4.2	5.3	4.8	3.3	3.9	3.9	4.2	2.9	3.55	1.71
Ser		6.9	5.8	4.1	4.6	3.8	4.2	5.1	2.9	5.21	2.64

a, sum of methionine and cysteine; b, sum of tyrosine and phenylalanine, according to the FAO protein reference pattern.

16 **Table 4. Effects of oral exposure to *Microcystis* spp. on animal growth and accumulation of microcystins (MC) in muscle.**

17

Animals		*Microcystis* spp. characteristics and MC concentrations	Dose	Duration	Effects on mortality and growth	MC Concentrations in muscle (ng/g dm)	References
Molluscs	zebra mussel	*M. aeruginosa* CCAP 1450/10, *M. aeruginosa* CCAP 1450/06	*M. aeruginosa* CCAP 1450/10: 2.7×10^6 cell/mL, *M. aeruginosa* CCAP 1450/06: 1.6×10^6 cell/mL	2 hours	clearance rate↓, filtration rate↓, ingestion rate↓, pseudofaecal production rate↑, absorption rate↓, and faecal production rate↓, net energy balance↓	NA	Juhel et al., 2006
Crustaceans	crayfish *Procambarus clarkii* juvenilebie	*M. aeruginosa* IZANCYA2, 11.3 µg MC/mg dm; *M. aeruginosa* IZANCYA6	4% cyanobacteria in diets	8 weeks	growth↓	NA	Vasconcelos et al., 2001
Fish	*Oreochromis mossambicus*	*M. aeruginosa* (95%), collected from Hartbeespoort Dam	-	15, 21 days	bm↓ on day 15, bm↑ on day 21	NA	de Moor and Scott, 1985
	roach (*Rutilus rutilus*)	*Microcystis* sp.	20% fish wet mass	10 days	growth rate↓	NA	Kamjunke et al., 2002ab
	common carp	*M. aeruginosa* (91.3%), collected from Naktong River, Korea, total MC (-RR, -LR, -YR, mainly -RR, 73.7%) 357.3 ± 26.8 µg MC/g dm	50 µg MC/kg bm/day	4 weeks	growth rate↓muscle	38.3 ± 12.3 ng MC-LReq/g wm	Li et al., 2004

gibel carp	*M. aeruginosa* (90%), collected from Lake Dianchi, China; 0.011% MC dm, 110 µg MC/g dm	15.15%, 29.79%, 44.69%, 59.58%, 74.48% cyanobacteria in diets; 39.12, 124.14, 174.5, 203.03, 228.92 ng MC/g diets	12 weeks	mortality↑, final bm↓, SGR↓, FCE↓, FR↑, ADCd↓, ADCp↓, ADCe↓	0.019, 0.03, 0.062, 0.147, 0.171	Zhao et al., 2006a
Nile tilapia	*M. aeruginosa* (90%), collected from Lake Dianchi, China; 0.14% MC dm, 1400 µg MC/g dm	1.19%, 2.34%, 3.51%, 4.68%, 5.85% cyanobacteria in diets; 1,253.52, 2,210.07, 3,363.02, 4,440.64, 5,460.06 ng MC/g diets	12 weeks	final bm↑, FR↑	0.77, 1.69, 2.59, 6.47, 14.62	Zhao et al., 2006b
hybrid tilapia	50% *M. aeruginosa*, 50% *M. wesenbergii*, collected from Lake Taihu, China	43.6% cyanobacteria in diets; 80 µg MC/g diets	30, 60 days	final bm↓, FR↑, SGR↓, FCE↓, PRE↓, ERE↓, ADCd↓, ADCp↓, ADCe↓	> 600	Dong et al., 2009
hybrid tilapia	*M. aeruginosa* (95%), collected from Lake Dianchi, China	50.8% cyanobacteria in diets; 410 µg MC/g diets	30, 60 days	final bm↓, FR↑, SGR↓, FCE↓, PRE↓, ERE↓, ADCd↓, ADCp↓, ADCe↓	> 600	Dong et al., 2009
female Nile tilapia	*M. aeruginosa* (90%)	5%, 20% cyanobacteria in diets; 4.92, 19.54 µg MC-LR/g diets	1, 7, 28 days	SGR↓, FCR↑	< detection limit of 3 ng/g fresh mass	Ziková et al., 2010
hybrid sturgeon	*M. aeruginosa* (90%), collected from Lake Dianchi, China; 0.81 mg MC/g dm	3%, 10%, 25% cyanobacteria in diets; 26.6, 78.82, 201.03 µg MC/g diets	24, 47 days	final bm↓, CF↓, FR↓, SGR↓, FCE↓, PRE↑↓, ADCd↓, ADCp↓	> 200	Dong et al., 2011
yellow catfish	*M. aeruginosa* (90%),	6.16%, 18.48%	30, 60	final bm↓, FR↓↑, SGR↓	> 100	Dong et al.,

		collected from Lake Taihu, China	cyanobacteria in diets; 32.3, 71.96 µg MC/g diets	days			2012
	Sacramento splittail	*Microcystis*, collected from San Francisco Estuary, USA, 872 mg MC-LR/kg dm	0.6%, 1.2%, 2.3% cyanobacteria in diets; 3.55, 9.14, 17.13 µg MC-LR/g diets	28 days	no significant changes of mortality, body mass, total length, CF	NA	Acuña et al., 2012b
	female and male threadfin shad	*Microcystis*, collected from San Francisco Estuary, USA, 872 mg MC-LR/kg dm	0.573%, 1.15% cyanobacteria in diets; 4.4, 10 µg MC/g diets	57 days	no significant changes of mortality, body mass, total length, SGR, CF↓	NA	Acuña et al., 2012a
	Indian major carp	*M. aeruginosa* (Kütz)	0.05%, 0.1%, 0.5% cyanobacteria in diets	90 days	SGR↑, FCR↓	NA	Das et al., 2013
	female goldfish	*M. aeruginosa* (90%) , collected from Lake Dianchi, China, 1.41 mg MC/g dm (-RR, -LR, -YR, 0.84, 0.50, 0.07 mg/g)	10, 20, 30, 40% cyanobacteria in diets	4, 8, 12, 16 weeks	final bm↑↓, final body lenth↑, final total lenth↑↓	> 40	Liang et al., 2015
	common carp larvae	*M. aeruginosa*, collected from Lalla Takerkoust reservoir, 976 µg MC-LR/g dm; *M. aeruginosa*, collected from Lalla Takerkoust reservoir, 968 µg MC-LR/g dm	0.1 g *M. aeruginosa*/kg dm, 60 ng MC/g dm, 0.06 ng MC/larva/day	12 days	larval mass↓, standard length↓, specific growth rate↓	NA	Ghazali et al., 2010
Amphibians	bullfrogs larval	*Microcystis incerta*	-	8 days	dry mass↓, snout-vent length (SVL)↓,		Pryor, 2003

	Xenopus laevis tadpoles	cyanobacterial biomass consisting mainly of *M. aeruginosa*	10, 50% *M. aeruginosa* in diets; 42.8, 187 μg MC-LR/g diets	1, 3, 7, 21 days	body width↓ body mass↓	NA	Ziková et al., 2013

↑, increased; ↓, decreased; bm, body mass; dm, dry mass; FR, feed rate; FCR, feed conversion ratio; FCE, feed conversion efficiency; SGR, specific growth rate; CF, condition factor; ADCd/ADCp/ADCe, apparent digestibility coefficient for dry matter/protein/energy; PRE, protein retention efficiency; ERE, energy retention efficiency. NA, not analyzed.

23 **Table 5. Effects of oral exposure to *Microcystis* spp. on molluscs.**

24

Molluscs	*Microcystis* spp. characteristics and MC concentrations	Dose	Duration	Toxic effects	References
mussel *M. galloprovincialis*	*M. aeruginosa* strain M6	1.5 $\times 10^5$ cells/mL	4 days	accumulation of MCs in the muscles, gill GST↑, digestive glands↑↓	Fernandes et al., 2009
zebra mussel	*M. aeruginosa* SAG 17.85, CCAP 1450/10 and CCAP 1450/06), SAG 48.80	10^4 cell/mL	7, 14, 21 days	haemocytes: no significant changes of cell viability, tail DNA↑	Juhel et al., 2007
freshwater snail *Bellamya aeruginosa*	*M. aeruginosa* FACHB-905	0.5×10^6 and 1 $\times 10^6$ cells/mL	15 days	hepatopancreas: accumulation of MCs, ACP↑, ALP↑, GST↑, ultrastructural damage	Zhu et al., 2011
pearl mussel	*M. aeruginosa* 905	40-60 μg MC-LR/L	15 days	accumulation of MC-LR: hepatopancreas > gonad > gill > muscle, 98 differentially expressed genes in the hepatopancreas	Yang et al., 2012
freshwater snail *Sinotaia histrica*	*M. ichthyoblabe* TAC95, 13.7 μg MC/mg dm	10^7 cells/mL	8 days	microstructural changes in hepatopancreas	Xie et al., 2014
zebra mussel	*M. aeruginosa* SAG 17.85, CCAP 1450/10, CCAP 1450/06), SAG 48.80	10^4 cell/mL	7, 14, 21 days	total hemocyte count↓, hyalinocyte percentage↓, granulocyte percentage↑, large basophilic hemocyte percentage↓, phagocytic rate↓, phagocytic index↓, lysozyme↑	Juhel et al., 2015
triangle sail mussel	*M. aeruginosa* FACHB-905	25, 50 mg/L of particulate organic	14 days	soft tissue: accumulation of MCs; haemolymph: GST↑, SOD↑, GPX↑, CAT↓,	Hu et al., 2015

		matters		GSH↑, lysozyme (LZM)↑	
triangle sail mussel	*M. aeruginosa* FACHB-905	1.8 ×10^7 cells/mL (50 mg/L)	3, 5, 7, 14, 21 days	accumulation of MCs in soft tissue, pathological alterations in the gills, digestive diverticula, and stomach	Wu et al., 2017
Female freshwater snail *Parafossarulus striatulus*	*M. aeruginosa*	10^7-10^8 cells/mL	8 weeks	129 down-regulated and 147 up-regulated unigenes in whole soft bodies	Qiao et al., 2018
triangle sail mussel	*M. aeruginosa* FACHB-905	5 ×10^5 cells/mL (1.4 mg/L), 5 ×10^6 cells/mL (14 mg/L)	1, 3, 7, and 14 days	digestive gland: cellulase↑, amylase↑, lipase↑; stomach: cellulase↑, amylase↑, lipase↑	Gu et al., 2019
marine mussel *Mytilus galloprovincialis*	*M. aeruginosa* LEGE 91094, 0.023 pg MC-LR/cell	10^5 cells/mL	14 days	no significant changes of filtration rate, number of byssal threads, whole body dry mass, total protein or glycogen content, 4 up-regulated and 3 down-regulated proteins in digestive gland	Oliveira et al., 2020

↑, increased; ↓, decreased; EC_{10}, EC_{25}, EC_{50}, Effective concentration 10%, 25%, 50%; LC_{10}, LC_{25}, LC_{50}, lethal concentration 10%, 25%, 50%; GST, glutathione S-transferase; ACP, acid phosphatase; ALP, alkaline phosphatase; SOD, superoxide dismutase; GPX, glutathione peroxidase; CAT, catalase; GSH, glutathione; LZM, lysozyme.

Table 6. Effects of oral exposure to *Microcystis* spp. on crustaceans.

Crustaceans	*Microcystis* sp. characteristics and MC concentrations	Dose	Duration	Toxic effects	References
crayfish *Procambarus clarkii*	*M. aeruginosa* IZANCYA2, 11.3 µg MC/mg dm	3% cyanobacteria in diets	2 weeks	accumulation of MCs in intestine and hepatopancreas	Vasconcelos et al., 2001
white shrimp postlarvae	*M. aeruginosa*, 0.01 pg MC-LR/mg dm	25, 208 cell/mL	48 hours	mortalities↑, histological lesion in antennal gland, gills, hepatopancreas, muscle, lymphoid organ, midgut, and dorsal cecum	Morales-Covarrubias et al., 2016
Microcystis sp. CYN06	*Microcystis sp.* CYN06, 1,400 µg MC/mg dm	6, 13, 33, 100 µg MC/kg dm diets	27 days	moults↓, specific growth rate↓, accumulation of MCs in hepatopancreas and muscle	Clearwater et al., 2014
male grapsoid crabs	*M. aeruginosa* NPJB, 230 µg MC/10^6 cells; *M. aeruginosa* NPDC1	4×10^5 cells/mL	1, 2, 3, 4, 5, 6, 7 weeks	hepatopancreas: accumulation of MCs, LPO↑, GSH↓, SOD↑, GST↑	Sabatini et al., 2015

↑, increased; ↓, decreased; dm, dry mass; LPO, lipid peroxidation; GSH, glutathione; SOD, superoxide dismutase; GST, glutathione S-transferase.

33 **Table 7. Effects of sub-chronic or chronic exposure to *Microcystis* spp. via oral route on fish.**

34

Fish	*Microcystis* sp. characteristics and MC concentrations	Dose	Duration	Toxic effects	References
common carp	*M. aeruginosa* (91.3%), collected from Naktong River, Korea, total MC (-RR, -LR, -YR, mainly -RR, 73.7%) 357.3 ± 26.8 µg MC/g dm	50 µg MC/kg bm/day	4 weeks	body color darker, growth rate↓; serum: ALT↑, AST↑; liver: ultrastructural changes; MC contents in organs of fishes: liver 261.0 ± 108.3 ng MC-LReq/g wm, muscle 38.3 ± 12.3 ng MC-LReq/g wm	Li et al., 2004
male Nile tilapia	*M. aeruginosa*, collected from River Guadiana, Portugal, 3230 µg MC-LR/g dm	about 1,200 µg MC-LR/kg bm/day, crushed *M. aeruginosa* cells	14 days	no significant changes of LPO, SOD, CAT, GPX, or GR in liver, kidney, gill	Jos et al., 2005
male Nile tilapia	*M. aeruginosa*, collected from River Guadiana, Portugal, 3340 µg MC-LR/g dm	about 1,200 µg MC-LR/kg bm/day, crushed *M. aeruginosa* cells	21 days	liver: LPO↑, SOD↑, CAT↑, GPX↑, GR↑; kidney: LPO↑, CAT↑, GPX↑, GR↑; gill: LPO↑, GPX↓	Jos et al., 2005
male Nile tilapia	*M. aeruginosa*, collected from River Guadiana, Portugal, 3340 µg MC-LR/g dm	about 1,200 µg MC-LR /kg bm/day, non-crushed *M. aeruginosa* cells	21 days	liver: SOD↑, CAT↑, GPX↑, GR↑; kidney: LPO↑, CAT↑, GPX↑, GR↑; gill: SOD↑, GPX↓	Jos et al., 2005
Chinese muddy loach	*Microcystis*, collected from Naktong River, Korea, 133 µg MC-RR/g dm	75 mg *Microcystis* kg bm/day (10 µg MC-RR/kg bm/day)	4 weeks	liver: SOD↑, CAT↑, GPX↑	Li et al., 2005
male tilapia	*M. aeruginosa*, collected from	about 1,200 µg	14 days	liver: micro- and ultra- structural changes, ACP↑;	Molina et al.,

	River Guadiana, Portugal, 3230 µg MC-LR/g dm	MC-LR/kg bm/day, crushed *M. aeruginosa* cells		kidney: micro- and ultra- structural changes, ACP↑, ALP↑; gill: protein↓	2005
male tilapia	*M. aeruginosa*, collected from River Guadiana, Portugal, 2647 µg MC-LR/g dm	about 1,200 µg MC-LR/kg bm/day, crushed *M. aeruginosa* cells	21 days	liver: micro- and ultrastructural changes, protein↓, ACP↑, ALP↑; kidney: micro- and ultra- structural changes, ACP↑, ALP↑; gill: microstructural changes, protein↓; intestine: microstructural changes	Molina et al., 2005
male tilapia	*M. aeruginosa*, collected from River Guadiana, Portugal, 2647 µg MC-LR/g dm	about 1,200 µg MC-LR/kg bm/day, non-crushed *M. aeruginosa* cells	21 days	liver: micro- and ultra- structural changes, protein↓, ACP↑, ALP↑; kidney: micro- and ultra- structural changes, ACP↑, ALP↑; gill: microstructural changes, protein↓; intestine: microstructural changes	Molina et al., 2005
female Nile tilapia	*M. aeruginosa* (90%)	5%, 20% cyanobacteria in diets; 4.92, 19.54 µg MC-LR/g dm diets	1, 7, 28 days	plasma: no significant changes of cortisol, glucose↑; liver: MCs accumulation, glycogen↓, no significant changes of GH and IGF- Ⅰ mRNAs; gill and muscle: < detection limit of 3 ng/g fresh mass	Ziková et al., 2010
male Nile tilapia	*M. aeruginosa* PCC7806, cultured in laboratory, 742 µg MC-LR/g dm	about 1,200 µg MC-LR/kg bm/day, crushed *M. aeruginosa* cells	21 days	liver: LPO↑, GPX↑, GST↑, GPX mRNA↑, GST mRNA↑, GST protein↑; kidney: LPO↑, GPX↓, GST mRNA↓, GST protein↓	Puerto et al., 2011
Sacramento splittail	*Microcystis*, collected from San Francisco Estuary, USA, 872 mg MC-LR/kg dm	6, 12, 23 g *Microcystis*/kg diets (120, 240, 460 mg *Microcystis*/kg bm/day); 3.55, 9.14, 17.13 mg	28 days	liver: PP2A↓, microstructural changes; muscle: RNA/DNA↓	Acuña et al., 2012b

		MC/kg diets (71, 182.8, 342.6 µg MC/kg bm/day)			
female and male threadfin shad	*Microcystis*, collected from San Francisco Estuary, USA, 872 mg/kg MC-LR dm	5.73, 11.5 g *Microcystis*/kg diets (171.9, 345 mg *Microcystis*/kg bm/day); 4.4, 10 mg MC/kg in diet (132, 300 µg MC/kg bm/day)	57 days	loss of muscle mass (cachexia), Fulton's condition factor↓, microstructural changes of liver in both femals and males and ovary in females, accumalation of MCs in gut, liver and kidney	Acuña et al., 2012a
male Nile tilapia	*M. aeruginosa*, 65.5 µg MC-LR/g dm	8, 16, 24 ($\times 10^4$ cells/mL)	7 days	after depuration for 30 days, liver: GSH↑, LDH↑, SOD↑, MDA↑, DNA fragmentation↑, DNA-protein crosslinks↑	Khairy et al., 2012
crucian carp	*Microcystis*, collected from Lake Dianchi, China, 1.41 mg MC/g dm (-RR, -LR, -YR: 0.84, 0.50, 0.07 mg/g)	20%, 40% cyanobacteria in diets	30 days	blood: nitroblue tetrazolium activity↑↓, lysozyme activity↑↓, macrophage bactericidal activity↑; head kidney: relative mass↑, micro- and ultra- structural changes, ; spleen: relative mass↑, micro- and ultra-structural changes	Qiao et al., 2013
Indian major carp	*M. aeruginosa* (Kütz)	0.5, 1, 5 g *M. aeruginosa*/kg dm diets	30, 60, 90 days	serum: total protein↑↓, albumin↑↓, globulin↑↓, albumin:globulin (A:G) ratio↓↑, lysozyme↑↓, bactericidal activity↓↑; blood: superoxide anion production↓↑	Das et al., 2013
Nile Tilapia	*M. aeruginosa*, collected from Thenneri Lake, India, 245.33 µg/g MC-LR dm	0.15, 0.30 g *M. aeruginosa*/fish/day (about 600, 1,200 µg MC-LR/kg bm/day)	14 days	swim erratically, fins hard, body pigmentation faded, whole body GST↑; liver: microstructural changes, protein↓, LPO↑, GST↑; gill: microstructural changes, GST↑; intestine:	Preeti et al., 2016

				microstructural changes	
Nile Tilapia	*M. aeruginosa*, collected from Mariout Lake, Egypt, 3500 µg/g MC-LR dm	about 1,200 µg MC-LR/kg bm/day	21 days	muscle: microstructural changes; serum: ALT↑, AST↑; liver: microstructural changes, MDA, GR, GPX, CAT; gill: microstructural changes	Abdel-Latif and Khashaba, 2017
blunt snout bream	*M. aeruginosa* (90%) , collected from Lake Dianchi, China, 1.41 mg MC/g dm (-RR, -LR, -YR: 0.84, 0.50, 0.07 mg/g)	15%, 30% cyanobacteria in diets	30 days	blood: white blood cells↓, phagocytosis activity↑↓, respiratory burst activity↑↓; head kidney: ultrastructural changes, sIgM mRNA↑↓, mIgD mRNA↓, sIgZ mRNA↓; spleen: sIgM mRNA↑↓, mIgD mRNA↑↓, sIgZ mRNA↑↓; MC contents in organs of fishes exposed to 30% cyanobacteria: head kidney 7.056 µg/g dm, liver 2.034 µg/g dm, intestine 1.933 µg/g dm, gonad 1.684 µg/g dm, spleen 1.254 µg/g dm	Xia et al., 2018

↑, increased; ↓, decreased; bm, body mass; dm, dry mass; PP, protein phosphatase; GH, growth hormone; IGF-I, insulin-like growth factor-I; LZM, lysozyme; LDH, lactate dehydrogenase; ACP, acid phosphatase; ALP, alkaline phosphatase; ALT, alanine amino-transferase; AST, aspartate amino-transferase; LPO, lipid peroxidation; MDA, malondialdehyde; CAT, catalase; SOD, superoxide dismutase; GSH, glutathione; GR, glutathione reductase; GPX, glutathione peroxidase; GST, glutathione S-transferase; mIgD, membrane-bound immunoglobulin class D; sIgM/sIgZ; secretory immunoglobulin class M/Z.

Table 8. Effects of oral exposure to *Microcystis* spp. on amphibians.

Amphibians	*Microcystis* spp. characteristics and MC concentrations	Dose	Duration	Toxic effects	References
bullfrog larvae	*Microcystis incerta*	-	8 days	dry mass↓, snout-vent length (SVL)↓, body width↓	Pryor, 2003
Xenopus laevis tadpoles	cyanobacterial biomass consisting mainly of *M. aeruginosa* collected	10, 50% *M. aeruginosa* in diets; 42.8, 187.0 µg/g diets	1, 3, 7, 21 days	body mass↓, aldosterone↓↑, corticosterone↓↑, LH mRNA in brain↑, mRNA of HSP 70, MDR in liver↑; no significant changes of mortality, developmental stages, mRNA of FSH in brain, mRNA of SOD, CAT, CYP 1A1, muGST, piGST in liver	Ziková et al., 2013
frog tadpoles	*M. aeruginosa* NIES-90, 2.768 µg MC-LR/mg dm	2, 4, 6 ($\times 10^6$ cells/mL)	7 days	no significant changes of survival	Zhang et al., 2012
frog tadpoles	*Microcystis sp.*	6 $\times 10^6$ cells/mL	3 days	bm↑	Zhang et al., 2012
frog tadpoles	*M. aeruginosa* NIES-90, 2.768 µg MC-LR/mg dm	-	7 days	bm↑	Zhang et al., 2012
Lithobates catesbeianus tadpoles	*M. aeruginosa* NPLJ4	10^5 cells/mL	16 days	liver, skeletal muscle, intestinal tract: no free MCs bioaccumulation, but serious histopathological damages	Júnior et al., 2018

↑, increased; ↓, decreased; bm, body mass; dm, dry mass; EC_{25}, Effective concentration 25%; LC_{25}, lethal concentration 25%; TI, teratogenic index; FSH, follicle stimulating hormone; LH, luteinizing hormone; CYP, cytochrome P450 monooxygenase; HSP, heat shock protein; MDR, multidrug resistance protein; CAT, catalase; GST, glutathione S-transferase; SOD, superoxide dismutase.

47 **Table 9. Effects of oral exposure to *Microcystis* spp. on mammals.**

48

Animals	*Microcystis* spp. characteristics and MC concentrations	Dose	Duration	Toxic effects	References
mice	*M. aeruginosa*, collected from Lake Mokoan, Australia	2.6 g algae/kg bm/day	21 days	50 % of mortality	Falconer et al., 1994
pigs	*M. aeruginosa*, collected from Lake Mokoan, Australia	80, 227, 374 mg algae/kg bm/day; 280, 796, 1,312 µg MC/kg bm; 100, 284, 469 µg MC-LReq/kg bm	44 days	plasma GGT↑, ALP↑, TBIL↑, albumin↓, microstructural changes in liver	Falconer et al., 1994; Chorus and Bartram, 1999
lactating Holstein-Friesian cows	*M. aeruginosa*	1.21±0.07 µg MC-LR/kg bm/day	3 weeks	no significant changes of blood bilirubin, GGT, GADH, AST, less than 2 ng MC-LR/L in milk	Orr et al., 2001
lactating Holstein-Friesian cows	*M. aeruginosa* PCC7806	0-13 µg MC-LR/kg bm/day	4 weeks	no significant changes of plasma TBIL, GGT, ALP, milk production↓, less than 0.2 µg MC-LR/L in milk	Feitz et al., 2002
beef cattle	*M. aeruginosa*	1.42±0.3 µg MC-LR/kg bm/day	28 days	no significant changes of water or feed intakes, body mass gain or relative growth rates, plasma GGT, GADH, AST, bilirubin, liver mass, 0.28-0.92 µg MC-LR/kg wm in liver	Orr et al., 2003
male Wistar albino rats	*Microcystis*, total MC 2.698 mg/g dw (-RR,	1% caynobacteria in diet; 3.2 mg MC/kg bm/day	28 days	serum CHOL↑, CREA↑, P↑, LIP↑, ALT↓, BIL↓, CHS↓, CD3-8+ lymphocytes in	Adamovsky et al., 2011

	-LR, -YR, 2 non identified: 1462, 1088, 96, 43, 9 µg/g)			peripheral blood↓, MC conjugates with cystein or GSH in liver	
male Wistar albino rats	*M. aeruginosa*, 2500 µg/g dm	1% caynobacteria in diets; 26,572 µg MC/kg diets (-LR, -RR, -YR, -LF, -LW: 8,829, 15,425, 872, 671, 775 µg/kg); 3,000 µg MC/kg bm/day	28 days	RBC count↓, MCV↓, MCH↑, MCHC↑, MCs around limit of detection (3-5 ng/g wm) in liver of 6/10 rats	Palikova et al., 2013
male Wistar albino rats	*M. aeruginosas*, 2500 µg/g dm	1% caynobacteria in diets; 26,572 µg MC/kg diets (-LR, -RR, -YR, -LF, -LW: 8,829, 15,425, 872, 671, 775 µg/kg); 3,033 µg MC/kg bm/day	28 days	microstructural changes in liver, hepato-, spleen-, and testes- somatic indexes↑, serun BIL↑, 5 ng MC-RR/g wm in liver of 6/10 rats	Adamovsky et al., 2013

↑, increased; ↓, decreased; bm, body mass; dm, dry mass; RBC, red blood cells; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentrations; BUN, blood urea nitrogen; ALP, alkaline phosphatase; ALT, alanine amino-transferase; AST, aspartate amino-transferase; LDH, lactate dehydrogenase; GDH, glutamate dehydrogenase; GADH, glyceraldehyde dehydrogenase; GGT, γ-glutamyl transpeptidase; GLU, glucose; BIL, bilirubin; TBIL, total bilirubin; DBIL, direct bilirubin; UBIL, unconjugated bilirubin; CHOL, cholesterol; CREA, creatine; P, phosphorus; LIP, lipases; CHS, cholinesterase; GSH, glutathione.

56 **Table 10. Effects of oral exposure to *Microcystis* spp. on birds.**
57

Birds	*Microcystis* spp. characteristics and MC concentrations	Dose	Duration	MC contents in organs	Toxic effects	References
male Japanese quails	*M. aeruginosa* 80%, M. viridis 15%, *M. flos-aquae* 5%, collected from Brno reservoir, Czech Republic, 373.3 µg MC/g dm (-RR, -YR, -LR, unidentified MC: 141.8, 33.7, 141.7, 56.1 µg/g)	0.2, 2.2, 22.4, 224.3 µg/kg bm/day	10, 30 days	MC contents in liver: 10 days > 30 days	plasma: LDH↑, glucose↓; liver: micro- and ultra- structural changes; testis: microstructural changes	Skocovska et al., 2007
male Japanese quails	*M. aeruginosa*, collected from Brno reservoir, Czech Republic, 373.3 µg MC/g dm (-RR, -YR, -LR, unidentified MC: 141.8, 33.7, 141.7, 56.1 µg/g)	0.2, 2.24, 22.46, 224.6 µg/kg bm/day	10, 30 days	MC contents in liver and muscle: 10 days > 30 days	liver: GST↑, GSH↑, GPX↑↓, GR↑, LPO↑; heart: EROD↑, GST↑, GSH↑, GR↑, LPO↑; brain: EROD↑, GSH↓↑, GPX↑, LPO↑	Pašková et al., 2008
male and female Japanese quails	*M. aeruginosa* 90% and *M. ichthyoblabe* 10%, collected from Musovska reservoir, Czech Republic	61.62 µg MC/bird/day (-RR, -YR, -LR: 26.54, 7.62, 27.39 µg)	8 weeks	NA	mass of eggs↓, mass of chicks at hatching and 14 days after hatching↓, fertilization rates (viability)↑, overall effect of hatching↑, number of 14-day old survivors per hen per day↑, plasma LDH↑	Damkova et al., 2009
male	*M. aeruginosa* 90%, *M.*	46.044 µg	10, 30	liver:	liver: microstructural changes	Pikula et al.,

Japanese quails	*ichthyoblabe* 10%	MC/bird/day (-RR, -YR, -LR: 15.36, 12.70, 17.98 µg); 210 µg/kg bm/day	days	39.94±17.75 ng MC/g wm		2010
male Japanese quails	*M. aeruginosa* 90% and *M. ichthyoblabe* 10%, collected from Musovska reservoir, Czech Republic	61.62 µg MC/bird/day (-RR, -YR, -LR: 26.54, 7.62, 27.39 µg)	8 weeks	NA	testis: microstructural changes, LPO↓, GPX↓, CAT↑	Damkova et al., 2011
male Japanese quails	*M. aeruginosa*	46 µg MC/bird/day (-RR, -YR, -LR: 15.36, 12.70, 17.98 µg), 210 µg/kg bm/day	30 days	liver: 39.9±17.7 ng MC/g wm	liver: GR↑; heart: GSH↑, GR↑	Paskova et al., 2011

↑, increased; ↓, decreased; bm, body mass; dm, dry mass; LDH, lactate dehydrogenase; EROD, 7-ethoxyresorufin O-deethylase; LPO, lipid peroxidation; CAT, catalase; GSH, glutathione; GR, glutathione reductase; GPX, glutathione peroxidase; GST, glutathione S-transferase.

Table 11. Protective role of antidotes versus toxic effects caused by acute oral exposure to *Microcystis* spp.

Animals	*Microcystis* spp. characteristics and MC concentrations	MC-LR dose and duration	Antidote dose and duration	Protective role	References
freshwater snail *Sinotaia histrica*	*M. ichthyoblabe* TAC95, 13.7 µg MC/mg dm	10^7 cells/mL, 8 days	Naringin, 0.581, 5.81 g/L; 1, 8 days	hepatopancreas: inhibition of MC-LR accumulation	Xie et al., 2014
male Nile tilapia	*M. aeruginosa*, 1350 µg MC-LR/g dm	about 2,400 µg MC-LR/kg bm, crushed *M. aeruginosa* cells; single dose; 24 hours	Trolox, a vitamin E analog; about 1.2, 4.2 mg/kg bm/day; 7 days	liver: LPO, protein oxidation, CAT, SOD; kidney: LPO, CAT, SOD, GR; gill: LPO, CAT, SOD, GPX	Prieto et al., 2008
male Nile tilapia	*M. aeruginosa*, 1350 µg MC-LR/g dm	about 2,400 µg MC-LR/kg bm, crushed *M. aeruginosa* cells; single dose; 24, 48, 72 hours	Trolox; about 4.2 mg/kg bm/day; 7 days	liver: micro- and ultra- structural changes, LPO, SOD, CAT, GPX, GR, GST, GSH/GSSG; kidney: micro- and ultra- structural changes, LPO, CAT, GPX, GR; gill: LPO, SOD, CAT, GPX, GR; heart and gastrointestinal tract: micro- and ultra- structural changes	Prieto et al., 2009
male Nile tilapia	*Microcystis*, collected from Guadiana River, Portugal, 2885 µg MC-LR/g dm	about 2,400 µg MC-LR/kg bm, crushed *Microcystis* cells; single dose; 24 hours	sodium selenite (Na_2SeO_3); 10, 18, 36 µg selenium (Se)/kg/day; 7	liver: micro- and ultra- structural changes, CAT, GPX, GST; kidney: micro- and ultra- structural changes, CAT, SOD, GPX; heart: micro- and ultra- structural changes;	Atencio et al., 2009

			days	gastrointestinal tract: micro- and ultra- structural changes	
male Nile tilapia	*Microcystis*, collected from Guadiana River, Portugal, 2885 µg MC-LR/g dm	about 2,400 µg MC-LR/kg bm, crushed *Microcystis* cells; single dose; 24 hours	N-acetylcysteine (NAC), a GSH precursor; 400, 880, 1,936 mg NAC/kg/day; 7 days	liver: LPO, protein oxidation, protein, CAT, GPX, GST, GSH/GSSG; kidney: LPO, CAT, SOD, GPX, GR	Puerto et al., 2009
male Nile tilapia	*Microcystis*, collected from Guadiana River, Portugal, 2885 µg MC-LR/g dm	about 2,400 µg MC-LR/kg bm, crushed *Microcystis* cells; single dose; 24 hours	NAC; 400, 880, 1,936 mg NAC/kg/day; 7 days	liver, kidney, heart, gastrointestinal tract, and gill: micro- and ultra-structural changes	Puerto et al., 2010

bm, body mass; dm, dry mass; LPO, lipid peroxidation; CAT, catalase; SOD, superoxide dismutase; GSH, glutathione; GSSG, oxidized glutathione; GR, glutathione reductase; GPX, glutathione peroxidase; GST, glutathione S-transferase.

Figure
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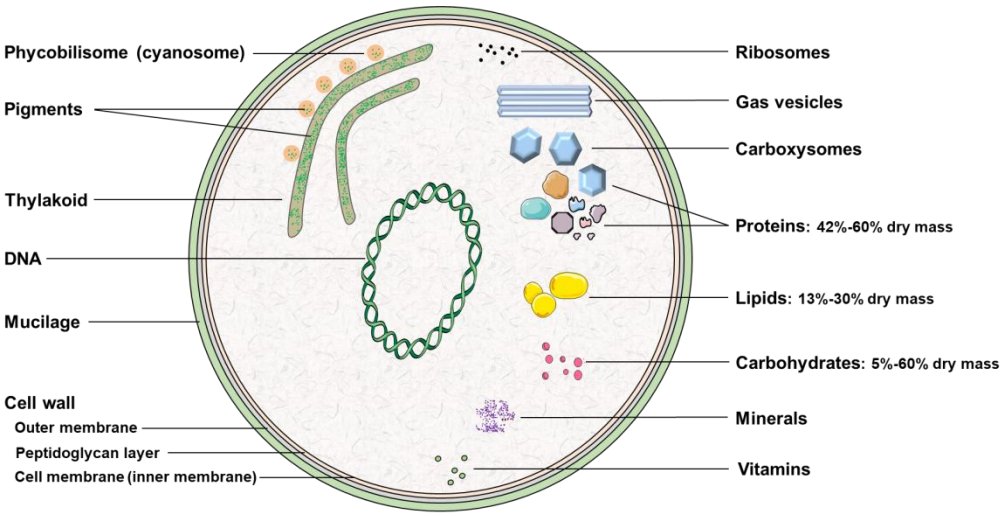

Figure 1. Schematic figure of *Microcystis* spp. cell illustrating chemical composition and nutritional values. The chemical composition and the nutrient value of *Microcystis* spp. vary considerably based on species, strains, environmental and growth conditions, the stage of cyanobacterial life cycle, geographic location and season, but *Microcystis* spp. are generally mostly composed of proteins, lipids and carbohydrates based on dry mass.

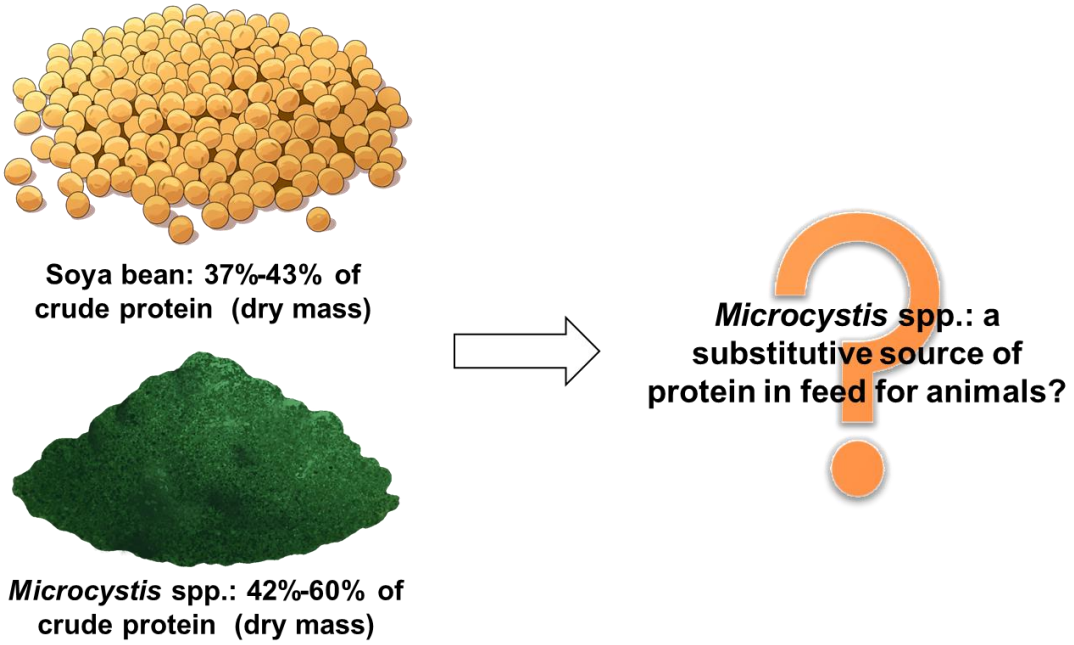

Figure 2. Potential use of *Microcystis* spp. as a substitutive source of protein in feed for animals. *Microcystis* spp. have been reported to contain 42%-60% crude protein (dry mass), which is close to the protein content of soybean (43%). Therefore, *Microcystis* spp. have been proposed to be used as protein sources for animal feeds.

Figure 3. Schematic procedure to transform biomass of*Microcystis* spp. from blooms to substitutive source of protein in feed for animals, which turn harmful blooms into goods (feed). *Microcystis* biomass is harvested from the environment, dried and powdered. Toxins, bioactive compounds and other harmful ingredients should be removed before the dried biomass is processed into feed.

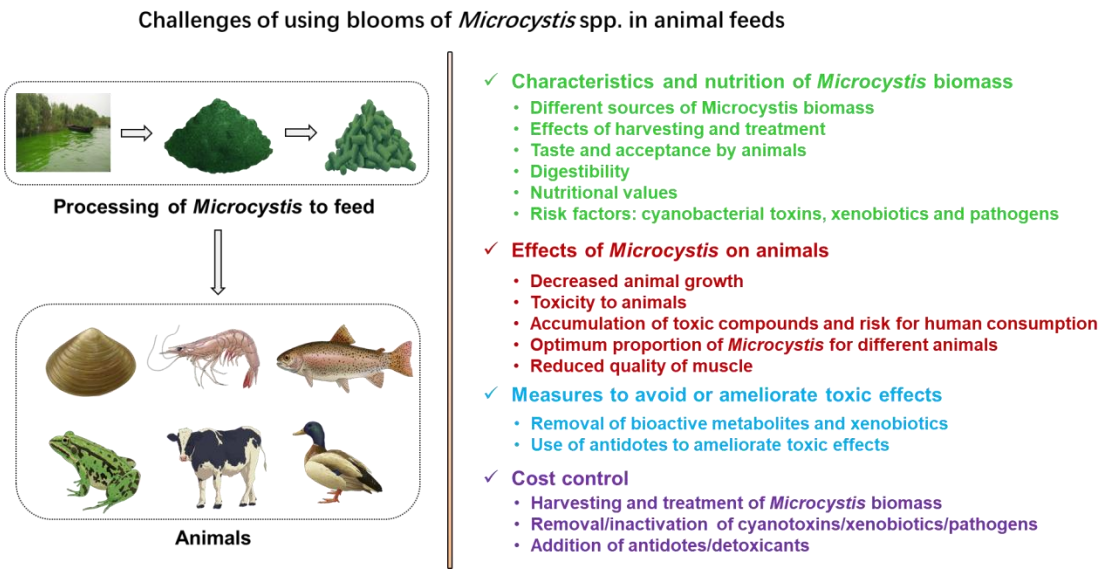

Figure 4. Overview of the challenges regarding the use of *Microcystis* biomass as component of animal feed. The potential pitfalls include the variable taxonomical and chemical composition of cyanobacterial biomasses, as well as documented adverse effects on broad spectrum of animals. The cost-benefit estimation of the use *Microcystis* as primary/main/supplemental ingredient in animal feed is also dependent on number of expensive actions to harvest and process the *Microcystis* biomass, ameliorate toxic effect of harmful compounds in the biomass.

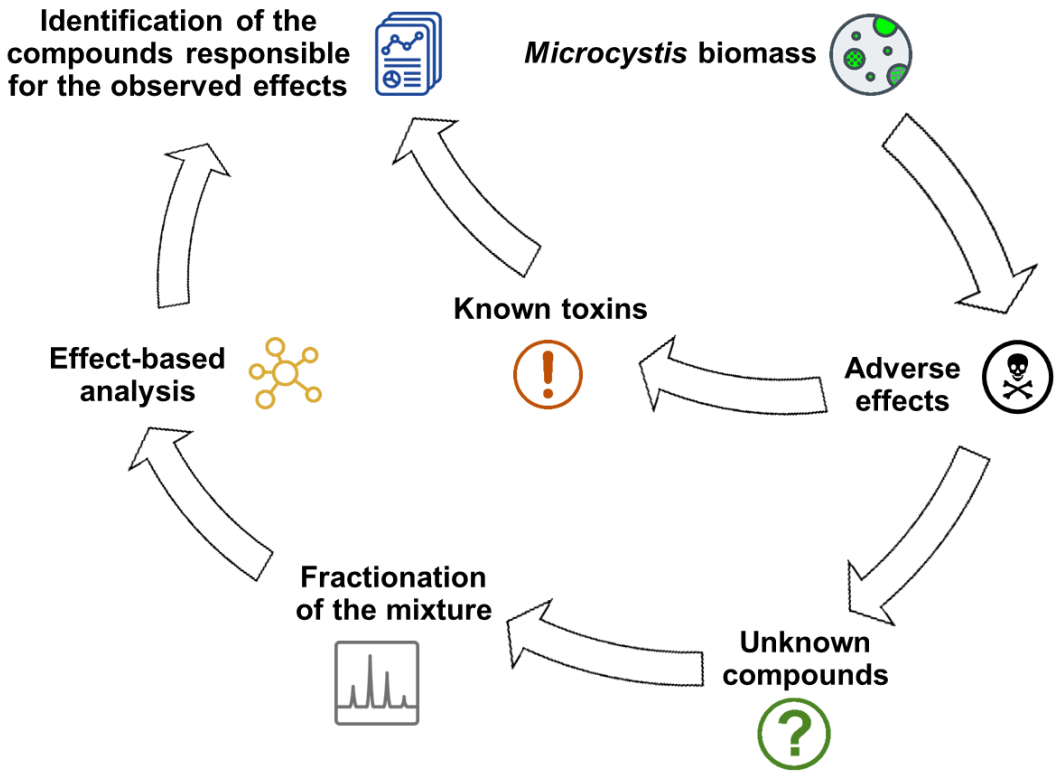

Figure 5. The proposed workflow to identify toxic compounds responsible for the adverse effects caused by biomas of *Microcystis* spp.). In scenarios, where the toxic effect is not associated with known toxins, effect-base analysis may be applied. To identify the compounds responsible for the observed effects, the complex *Microcystis* biomass has to be fractionized and the individual fractions tested. Positive fraction can be further sub-fractioned to obtain individual compound or set of chemically similar compounds.

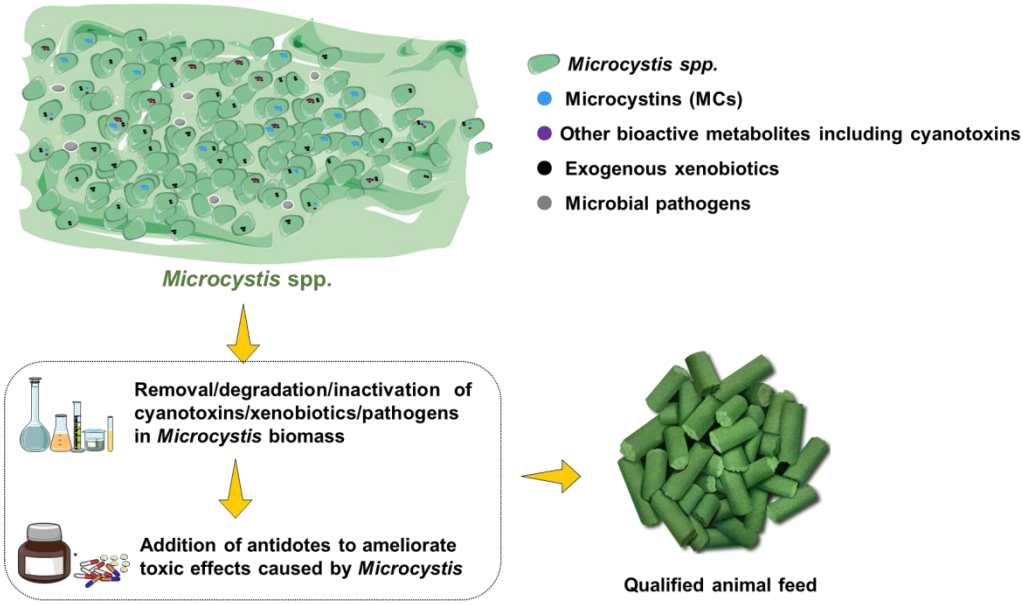

Figure 6. Measures to avoid or ameliorate toxic effects caused by *Microcystis* spp. *Microcystis* has some limitations for use in animal feed: 1) microcystin (MC)-producing and non-MC-producing strains co-exist in a bloom; 2) *Microcystis* can produce other bio-active metabolites beyond MCs; 3) *Microcystis* can also bioaccumulate exogenous xenobiotics in aquatic environments, including heavy metals, polycyclic aromatic hydrocarbons (PAHs), organochlorine pesticides (OCPs); 4) microbial pathogens can occur in *Microcystis* blooms. Therefore, cyanotoxins/xenobiotics/pathogens in *Microcystis* biomass should be removed/degraded/inactivated sufficiently to assure safety for use as a primary/main/supplemental ingredient in animal feed. As an ameliorative measure, antidotes/detoxicants can be used to avoid/reduce the toxic effects.

Declaration of Interest Statement

None.