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Protective effects of Spirulina (*Arthrospira maxima*) against toxicity induced by cadmium in *Xenopus laevis*

Itzayana Pérez-Alvarez [a], Hariz Islas-Flores [a,*], Leobardo Manuel Gómez-Oliván [a], Livier Mireya Sánchez-Aceves [a], Germán Chamorro-Cevallos [b]

[a] Laboratorio de Toxicología Ambiental, Facultad de Química, Universidad Autónoma del Estado de México, Paseo Colon intersección Paseo Tollocan s/n, Col. Residencial Colon, 50120 Toluca, Estado de México, Mexico

[b] Departamento de Farmacia, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Unidad Profesional Adolfo López Mateos, Av. Wilfrido Massieu Esq. Cda. Miguel Stampa S/N, Delegación Gustavo a. Madero, México, DF C.P. 07738, Mexico

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ABSTRACT

Spirulina (*Arthrospira maxima*) has been recognized as a superfood and nutraceutical by its high nutritional value and the benefits of its consumption; it is an important source of lipids, proteins, vitamins, minerals, and antioxidants. It is known that spirulina has positive effects on the toxicity induced by pharmaceuticals and metals. Heavy metals such as cadmium, frequently used in industrial activities, are continuously detected in water bodies and can generate adverse effects on aquatic organisms even at low concentrations. This study aimed to evaluate the protective effect of spirulina (*Arthrospira maxima*) against the toxic effects induced by cadmium in the early life stages of *Xenopus laevis*.

Twenty *Xenopus laevis* embryos were exposed to five different treatments on triplicate, control, cadmium ($CdCl_2$ 24.5 μg L^{-1}) and three spirulina mixtures Cd + S 1 (24.5 μg L^{-1} $CdCl_2$ + 2 mg L^{-1} spirulina), Cd + S 2 (24.5 μg L^{-1} $CdCl_2$ + 2 mg L^{-1} spirulina), Cd + S 3 (24.5 μg L^{-1} $CdCl_2$ + 10 mg L^{-1} spirulina); after 96 h of exposure: Malformations, mortality and length were evaluated; also, after 192 h, lipid peroxidation (LPX), superoxide dismutase (SOD) and catalase (CAT) were determined.

All spirulina treatments decreased mortality from 34 to 50% and reduced malformations on incidence from 36 to 68%. Treatment Cd + S 3 decreased growth inhibition significantly. Spirulina treatment Cd + S 2 decreased lipidic peroxidation and antioxidant activity; these results suggest that spirulina (*Arthrospira maxima*) can decrease the mortality, frequency of malformations, the severity of malformations, growth inhibition, and oxidative damage induced by cadmium in *Xenopus laevis* embryos.

1. Introduction

Aquatic pollution has become one of the most critical problems worldwide; water bodies are continuously overloaded with pollutants such as pesticides, pharmaceuticals, households, personal care products, industrial additives, and heavy metals; found in urban, hospital, and industrial wastes (Hayati et al., 2017). Heavy metals are released in to aquatic environments daily from atmospheric depositions, geologic processes or through anthropogenic activities (Authman, 2015). Metallic elements including iron, arsenic, lead, and cadmium have shown to be toxic even at low concentrations (Al-homaidan et al., 2015; Marin et al., 2015).

Cadmium (Cd) is one of the most toxic metals occurring in water bodies, either naturally or as an anthropogenic pollutant arising from various sources, mainly industrial and agricultural activities (Järup and Åkesson, 2009) and has been detected in water in variable concentrations from 0.0001 to 0.0354 mg L^{-1} and in soils 0.019–0.143 mg kg^{-1} (Rangkadilok et al., 2015; Salnikova et al., 2018). Cadmium is a nonessential, highly toxic metal for the aquatic biota with weak hydrophilicity, high persistence, low rate of excretion, and a robust bioaccumulative behavior, mainly in kidneys, liver, and reproductive organs of many aquatic species (Farag et al., 2015; Hu et al., 2015).

Since cadmium shows a long environmental half-life, it has been related to severe deleterious effects in aquatic organisms and wildlife (Authman, 2015; Avallone et al., 2015; Kasherwani et al., 2009; Chan and Cheng, 2003; Ercal et al., 2001). *In vitro* cadmium stimulates the

* Corresponding author.
E-mail address: hislasf@uaemex.mx (H. Islas-Flores).

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inhibition of mitochondrial respiratory chain complexes triggering the increase of reactive oxygen species (ROS) (Wang et al., 2004) as well as the induction of DNA impair and apoptotic response in zebrafish embryos (Cambier et al., 2010; Chan and Cheng, 2003). This metal inhibits the DNA repairing system and interferes with reduced glutathione (GSH) involved in the oxidative stress response and redox cycling (Giaginis et al., 2006).

Mouchet et al., 2006 evaluated the potentially toxic effects of Cd in *Xenopus laevis* larvae founding that at environmental concentrations 2, 10, 30 μg L^{-1}; this metal causes genotoxicity, increases the production of metallothionein; and also up-regulates *Sod* cytoplasmic and mitochondrial genes, as well as mitochondrial metabolism (*Cox—I*) and detoxification (*mt1*), which is responsible for detoxification processes. Sharma and Patiño (2009) demonstrated that Cd levels up to 860 μg L^{-1} delay metamorphosis in *Xenopus laevis* tadpoles. Cd promotes the production of free radicals *via* peroxide induction, enhancing oxidative cell biomarkers in *Meretrix meretrix* exposed to 1.5, 3, 6, and 12 mg L^{-1} (Xia et al., 2016). On the other hand, Wu et al. (2017a, 2017b) found that embryos of *Bufo gargarizans* exposed to 5 up to 500 μg L^{-1} of Cd shown inhibition of growth and development and induction of morphological malformations mainly axial and fin flexures, abdominal edema, and delayed growth. This study also showed that cadmium might have an endocrine-disrupting action in embryos as it was capable of changing the mRNA expressions of TRα, *Dio*2, and *Dio*3, genes related to developmental features.

Exogenous supplementation with antioxidant molecules might have an essential role in depleting oxidative damage caused by heavy metals on mammals and aquatic organisms (Bashandy et al., 2016; Rodríguez-Sánchez et al., 2012; Szuroczki et al., 2016).

Spirulina (*Arthrospira maxima*) is an edible free-floating filamentous blue-green microalgae that belong to the cyanobacteria class with photosynthetic activity (Deng and Chow, 2010; Kulshreshtha et al., 2008). It has multiple pharmacological and nutraceutical properties as it is highly rich in protein (ranges between 60 and 70%), γ-linoleic and linolenic acids, β-carotene, and vitamins (Castro-García et al., 2018; Kulshreshtha et al., 2008; Mühling et al., 2005; Rodríguez-Sánchez et al., 2012). Phytochemicals and phycobiliproteins as chlorophyll α, zeaxanthin, C-phycocyanin, cryptoxanthin, and allophycocyanin (Yan et al., 2011; Kumar et al., 2016) also found in significant amounts in the *Arthrospira* genus. Due to the presence of bioactive compounds found in spirulina, multiple potential health benefits associated with consumption have been reported (Kumar et al., 2016; de la Jara et al., 2018). Spirulina is known to have anti-inflammatory (Aladaileh et al., 2020; Deng and Chow, 2010; Joventino et al., 2012), antioxidant (Abdelkhalek and Ghazy, 2014; Ferruzzi and Blakeslee, 2007; Nasirian et al., 2018; Rodríguez-Sánchez et al., 2012), neuroprotective (Lima et al., 2017; Pérez-Juárez et al., 2016), antimutagenic properties as well as properties for preventing the enhancement of oxidative stress and cellular damage induced by heavy metals (Castro-García et al., 2018; Rodríguez-Sánchez et al., 2012).

Studies carried out with the *Arthrospira* genus have evidenced the potential benefits of these microalgae for aquatic organisms exposed to environmental toxicants. Abdelkhalek et al. (2017) demonstrated the protective role of supplementary spirulina against oxidative stress induced by diazinon in Nile tilapia, finding that spirulina improved liver and kidney functions showing a significant enhancement of antioxidant activity. Sayed and Authman (2018) studied the potential ameliorative influence of *Spirulina platensis* (SP) in *Clarias gariepinus* exposed to sodium dodecyl sulfate (SDS), founding that the SP supplementation restored biochemical and genetical variations induced by the SDS, including hepatic and renal dysfunctions, disruption in enzymatic and non-enzymatic antioxidants and apoptosis in erythrocytes among others. On the other hand, Mahmoud et al. (2018) demonstrated that feeding supplementation with Spirulina (*Arthrospira platensis*) improved growth performance, feed utilization, immune response, and oxidative stress response in a trial performed with *Oreochromis niloticus* challenged with *Pseudomonas fluorescence*.

The presence of pollutants in the aquatic environment stimulates a variety of toxicity mechanisms, including oxidative stress, known as an imbalance between prooxidant species and antioxidant defenses due to the overproduction of reactive oxygen species (ROS), and the depletion of protective intracellular mechanisms (Abdelkhalek and Ghazy, 2014; Ganesan et al., 2016). The quantification of biomarkers has been extensively used to determine the effects of ROS in main biomolecules like lipids, proteins, and DNA in organisms exposed to environmental toxicants (Valavanidis et al., 2006).

The impact of oxidative stress on embryotoxic development and teratogenic effects have been well documented (Adeyemi et al., 2015; Ganesan et al., 2016; Wu et al., 2017a, 2017b; Sant et al., 2017; Timme-Laragy et al., 2018). Redox status plays an essential role during critical embryonic cellular processes, including proliferation, differentiation, signaling, and apoptosis (Dennery, 2007). ROS is crucial to normal embryo development, cellular signaling, control of cellular mechanisms, and second messengers by regulating key transcription factors (Dennery, 2007; Wu et al., 2017a; Xia et al., 2016). An increase in ROS produces not only oxidative stress but also other toxicity mechanisms that may result in developmental alterations, functional abnormalities, congenital malformations, and embryo death in aquatic organisms (Gilbert-Barness, 2010; Pašková et al., 2011).

Xenopus laevis African clawed frog is an important model organism for vertebrate development as it has been used as a suitable bioindicator due to its fully aquatic environment, short reproductive cycle, and the throughout molecular and cellular characterization of its embryogenesis (Brausch et al., 2010; Cardoso-Vera et al., 2017; Pike et al., 2019; Robert and Ohta, 2009). The fertilization and development occur externally, allowing direct and easy observation during embryogenesis; the embryo development takes place in a short time within 96 hour post-fertilization (hpf), manipulations are simplified as the eggs have a relatively large size (Peuchen et al., 2016). The ecotoxicological importance of *Xenopus laevis* on the evaluation of embryotoxicity and teratogenic malformation using the FETAX assay has been well established (Bonfanti et al., 2004; Mouchet et al., 2006; Pérez-Alvarez et al., 2018). The frog embryo teratogenesis assay- *Xenopus* FETAX method constitutes a valuable tool to evaluate the developmental toxicity hazards of pure chemical agents and mixtures as it determines developmental endpoints, including lethality, morphologic alterations, and minimum growth-inhibition concentration (Cardoso-Vera et al., 2017; Hu et al., 2015; Isidori et al., 2016). Amphibians are considered beneficial organisms for assessing toxic effects exerted by various pollutants (Hopkins, 2007).

In the present study, antioxidant effects of spirulina (*Arthrospira maxima*) were evaluated against cadmium-induced toxicity in early life stages of *Xenopus laevis*; throughout the evaluation of mortality, malformations, growth inhibition, lipoperoxidation, and antioxidant activity of superoxide dismutase and catalase.

2. Materials and methods

2.1. Test substances

Cadmium chloride $CdCl_2$ (CAS# 10108-64-2, >99% purity), 183.32 Da, Sigma-Aldrich (St. Louis, MO), *Arthrospira maxima* dried and as the powder was purchased from a local supplier AEH Spiral Spring, Mexico. All other chemical reagents were acquired from Sigma-Aldrich (St. Louis, MO).

2.2. Test organisms

All procedures were performed following the ethical protocols of care, use, and management of the species used in the testing of the Universidad Autonóma del Estado de México. The specifications mentioned in the corresponding Official Mexican Standards were also considered (NOM-062-ZOO- 1999, Technical specifications for the

Table 1
Exposure groups.

Exposure solution	Mixtures
Control	FETAX medium
Spirulina	10 mg L^{-1} of *Arthrospira maxima*
Cadmium	24.5 μg L^{-1} of $CdCl_2$
Cd + S 1	24.5 μg L^{-1} of $CdCl_2$ plus 2 mg L^{-1} of *Arthrospira maxima*
Cd + S 2	24.5 μg L^{-1} of $CdCl_2$ plus 4 mg L^{-1} of *Arthrospira maxima*
Cd + S 3	24.5 μg L^{-1} of $CdCl_2$ plus 10 mg L^{-1} of *Arthrospira maxima*

production, care, and use of laboratory animals).

Adult *Xenopus laevis* were obtained from the aquaculture center "Aquanimals", located in Queretaro, Mexico. The selection criteria for males were 8 to 10 cm long and two years old, and females 10 to 12.5 cm long and three years old. Females were identified by the presence of cloacal labia and a larger size. *X. laevis* were acclimated in natural water, with the following conditions: pH 6.5 to 9, total organic carbon <10 mg L^{-1}, alkalinity, and hardness by determining $CaCO_3$ 16 to 400 mg L^{-1}; parameters were determined monthly. Organisms were kept in a light-protected room with photoperiods 12 h light/12 h darkness. Males and females stayed separate in 60 L fish tanks filled to 80% capacity, at 21 ± 3 °C. They were fed three times a week *ad libitum* with *Chrisotoma* sp. (0.5 ± 0.3 cm in length) or commercial food pellets NUTRIPEC® Purina.

2.3. FETAX assay

This study was carried out following the standard guidelines of the American Society for Testing Materials, ASTM (ASTM E1439 – 12, 2019). All experiments were performed in triplicate.

2.3.1. FETAX medium

FETAX medium was prepared by dissolving 625 mg NaCl, 96 mg $NaHCO_3$, 30 mg KCl, 15 mg $CaCl_2$, 60 mg $CaSO_4$ C_2H_2O, and 75 mg $MgSO_4$ in a final volume of 1 L distilled water; final pH was 7.6–7.9. All reagents were purchased from Sigma-Aldrich (St. Louis, MO).

2.3.2. Breeding induction

One male and one female were placed in a 40-L aquarium with a plastic mesh suspended roughly 3 cm over the bottom, into which oocytes could be laid. Aquarium sides were opaque, and the water temperature was 20 ± 2 °C, with a 12 h light/12 h darkness photoperiod.

Human chorionic gonadotropin hormone (CHORAGON®, Ferring) was dissolved in a sterile NaCl 0.9% solution; subsequently, males and females were administered in the dorsal lymph sac with 300 IU and 700 IU respectively, using a 1 mL hypodermic syringes fitted with long 26-gauge needles.

2.3.3. Embryo selection

The next day morning, fish tanks were inspected for oviposition. Fertilized oocytes were extracted from the tank using the nylon mesh and sterile Pasteur pipettes and were placed in separate containers for examination under a Zeiss Stemi 305 stereoscopic microscope to select those in mid blastula to early gastrula stage (stage 8–11).

2.3.4. Preparation of cadmium, spirulina, mixtures, and control solutions

A stock solution of cadmium and another of spirulina was prepared daily by dissolving 1 and 10 mg, respectively, in 1 L of FETAX medium. Negative control was exposed only to the FETAX medium. Mixtures were prepared by dissolving 2, 4, and 10 mg of spirulina in a 24.5 μg L^{-1} of Cd solution. The entire procedure was done under a laminar flow hood. The stock solutions were stored in amber glass bottles at 4 °C.

Cadmium concentration (24.5 μg L^{-1}) was selected based on a previous exposure described on Section 2.3.5.1. Spirulina (*Arthrospira maxima*) concentrations for the mixtures (2, 4, and 10 mg L^{-1}) were selected based on experimental procedures, due there are no concentrations reported to be used in embryonic stages of development of aquatic organisms or amphibians yet; for the exposure of the control of spirulina alone, we decided to use the highest concentration to visualize the maximum effect. Table 1 shows the exposed groups.

2.3.5. Exposure

2.3.5.1. Cadmium. Under a laminar flow hood, 10 mL of each cadmium solution was added in previously labeled 50-mm Petri dishes, embryos with spherical shape, homogeneous cell division, and mid blastula to early gastrula stage (stage 8–11) were collected using dissecting forceps and a stereoscopic microscope. Twenty oocytes were placed in each petri dish containing the different cadmium solutions for exposed (0.61, 2.45, 4.91, 9.82, 19.63, and 38.34 mg L^{-1}) and control group (FETAX medium) all on triplicate, Petri dishes were kept in the incubator at 21 ± 2 °C in the dark for 96 h and the attainment of stage 46 in controls.

2.3.5.2. Mixtures cadmium + spirulina (Arthrospira maxima). Under a laminar flow hood, 10 mL of each solution were added in previously labeled 50-mm Petri dishes. Embryos with a spherical shape, homogeneous cell division, and in medium blastula stage (stage 8) were collected using dissecting forceps and a stereoscopic microscope, 20 oocytes were placed in each Petri dish containing different mixtures (Cd + S 1, Cd + S 2, Cd + S 3) and control group, all on triplicate; were kept in the incubator at 21 ± 2 °C in the dark for 96 h and the attainment of stage 46 in controls.

2.3.6. Culture monitoring

Control, cadmium, and spirulina mixtures solutions were replaced daily under the laminar flow hood. To this end, 10 mL of each test concentration were added in new and previously labeled, sterile 50-mm Petri dishes, maintained for 1 h 30 min at room temperature to ensure that the medium was 20 ± 2 °C before embryos were transferred. Each 24 h, live embryos were transferred to new Petri dishes, using the stereoscopic microscope and Pasteur pipettes to separate them from the dead embryos. A daily record was taken, the number of dead embryos and precipitates (if any) in each culture were documented.

2.3.7. Examination of larvae

At 96 h of exposure, larvae were checked for swimming; if not swimming, this was noted in a developmental parameter sheet. Precipitates (if any) were also registered, as well as the number of dead larvae.

X. laevis larvae were euthanized by placing them in a petri dish containing a 0.06% MS-222 solution (lethal dose). Each larva was then measured straight from head to tail using Zen Blue Zeiss software, values were registered in the developmental parameter sheet. Next, each larva was evaluated under the microscope, fitted with a Zeiss Axiocam 5s camera to identify malformations according to Atlas of Abnormalities (Bantle et al., 1991) and other resources. Cadmium LC_{50} and LOAEL were determined to evaluate spirulina and cadmium mixtures. After examination, larvae were disposed of following institutional standards for the elimination of biological samples.

2.4. Oxidative stress evaluation

FETAX medium was prepared, and embryos were exposed to the stated mixtures and control on triplicate, using the procedures described in Sections 2.3.1 to 2.3.5. *Xenopus laevis* start feeding at stage 45 (Nieuwkoop and Faber, 1994), and 24 h after fertilization, the activity of SOD and CAT are developed; after 48 h, GSH is activated, and the activity of other enzymes increase (Rizzo et al., 2007). If the exposure time is extended further than stage 50, the alimentation must be enriched (Ishibashi and Amaya, 2020), which would involve a change in

experimental conditions. For this reason, organisms were exposed for 192 h, or Nieuwkoop stage 57, to ensure that they were feeding and developed their antioxidant enzymes.

Afterward, they were treated as mentioned in Section 2.3.6, then larvae in each plate were weighed and mechanically homogenized 1:4 (w/v) with a 4 °C phosphate buffer solution (pH 7.2), then centrifuged at 2500 rpm for 15 min. All determinations were made on the supernatant.

2.4.1. Determination of lipid peroxidation (LPX)

Determination was made by the Buege and Aust (1978) method. 1 mL Tris-HCl buffer solution (150 mM) pH 7.4 was added to 500 mL of supernatant. The resulting sample was incubated at 37 °C for 30 min, then 2 mL of 0.38% thiobarbituric acid (TBA) (Fluka, Sigma-Aldrich, Toluca) in 15% TCA were added before incubation at 37 °C for 45 min. Then samples were centrifuged at 12,500 rpm and −4 °C for 15 min; subsequently, absorbance was determined at 535 nm. Results were expressed as mM malondialdehyde (MDA)/mg protein, using the molar extinction coefficient (MEC) of 1.56×10^5/M/cm.

2.4.2. Determination of superoxide dismutase (SOD) activity

SOD activity was determined by Boehringer's (1987) method. Samples were delipidated by the addition of 30 μL chloroform and 50 μL methanol to 100 μL of serum. The mixture was shaken for 1 min and centrifuged for 15 min at 6000 rpm, and the supernatant was kept.

In a quartz cuvette, 50 μL of distilled water were mixed with 1.4 mL Tris-HCl buffer solution (50 mM, pH 8.20) and 25 mL EDTA solution (1 mM), subsequently supplementing with 25 μL of a pyrogallol solution (0.124 mM). Absorbance was read at 0, 10, and 60 s, at 420 nm. The assay was performed on triplicate, and the difference was taken as the optical density (OD) of the blank (DODblank), representing the difference in ODs for the non-inhibited reaction.

For each sample, 50 μL of supernatant was mixed in a quartz cuvette with 1.4 mL of Tris-HCl buffer solution and 25 mL of EDTA solution. Next, 25 μL pyrogallol was added, and absorbance was read at 410 nm, at 0, 10, and 60 s. The following formula derived results:

$$|[(\text{Mean DODsample} \times 100)/(\text{Mean DODblank})] - 100| \times 0.6$$

where:

DODsample = difference in optical densities in the sample
DODblank = difference in optical densities in the blank

2.4.3. Determination of catalase (CAT) activity

CAT activity was determined by the Radi et al. (1991) method. 1 mL of isolation buffer solution [0.3 M sucrose (Vetec, Sigma-Aldrich, St. Louis), 1 mM EDTA, 5 mM HEPES, 5 mM KH_2PO_4 (Vetec)] was added to 20 μL of supernatant, plus 0.2 mL of 20 mM H_2O_2 solution (Vetec). Absorbance was read at 240 nm, at 0 and 60 s, and CAT activity per minute was estimated using the MEC of H_2O_2 (0.093 mM/cm).

2.5. Statistical analysis

The data were analyzed using the software Stat graphics Centurion XVI. All the results were expressed as the mean of three experiments performed under the same conditions. Data normality and homoscedasticity were verified by Shapiro-Wilk and Levene tests, respectively. In the FETAX assay, acute toxicity was assessed by determining the 96-h LC50 and their respective 95% confidence limit (C.L.) using probit analysis. To calculate the LOAEL, we performed a Dunnett's test ($P < 0.05$). To determine the differences in the number of death/malformed embryos and growth, each larva was measured from head-to-tail, and the mean values were compared by one-way analysis (ANOVA) and Fisher's multiple comparisons ($P < 0.05$). Lipid peroxidation and enzymatic activity (SOD and CAT) were analyzed by one-way analysis of variance (ANOVA) and Fisher's multiple comparisons ($P < 0.05$).

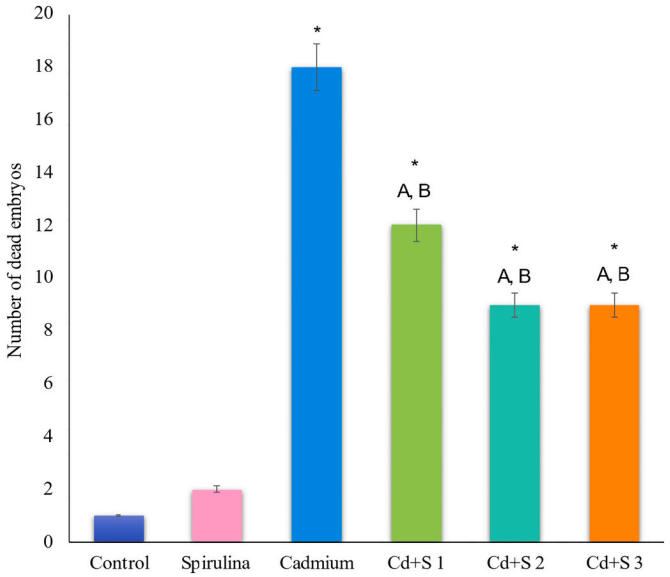

Fig. 1. Number of *Xenopus laevis* dead embryos after 96 h exposed to control, spirulina 10 mg L^{-1}, cadmium 24.5 μg L^{-1}, Cd + S 1 (cadmium 24.5 μg L^{-1} + spirulina 2 mg L^{-1}) Cd + S 2 (cadmium 24.5 μg L^{-1} + spirulina 4 mg L^{-1}), Cd + S 3 (cadmium 24.5 μg L^{-1} + spirulina 10 mg L^{-1}), significant differences relative to: (*) control, (A) spirulina, (B) cadmium (One-way ANOVA and Fisher's test, $P < 0.05$).

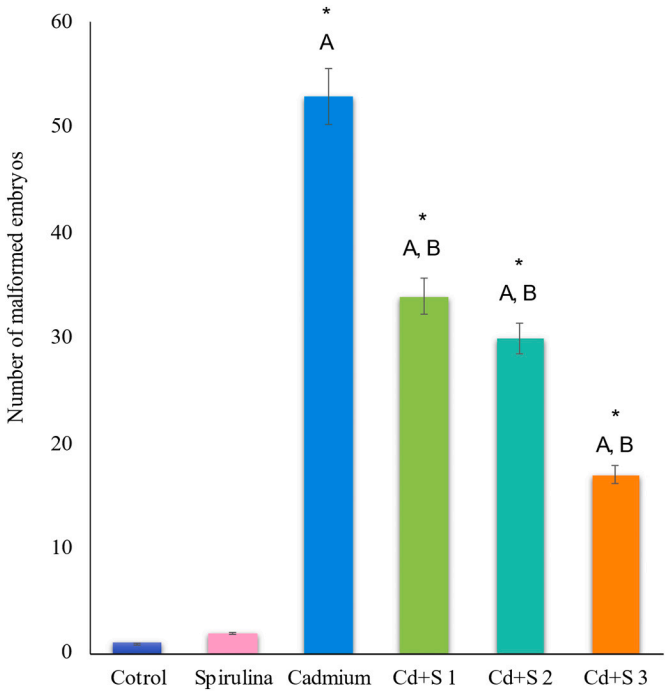

Fig. 2. Number of *Xenopus laevis* embryos with malformations after 96 h exposed to control, spirulina 10 mg L^{-1}, cadmium 24.5 μg L^{-1}, Cd + S 1 (cadmium 24.5 μg L^{-1} + spirulina 2 mg L^{-1}) Cd + S 2 (cadmium 24.5 μg L^{-1} + spirulina 4 mg L^{-1}), Cd + S 3 (cadmium 24.5 μg L^{-1} + spirulina 10 mg L^{-1}), significant differences relative to: (*) control, (A) spirulina, (B) cadmium (One-way ANOVA and Fisher's test, $P < 0.05$).

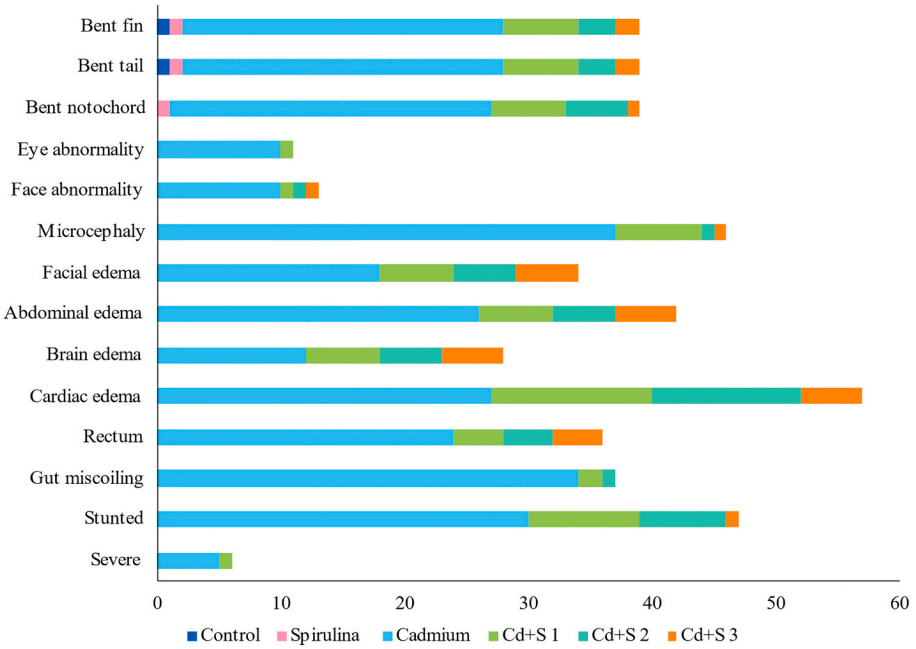

Fig. 3. Histogram of frequency for malformations induced in *Xenopus laevis* embryos by the exposure to control, spirulina 10 mg L^{-1}, cadmium 24.5 μg L^{-1}, Cd + S 1 (24.5 μg L^{-1}, + spirulina 2 mg L^{-1}) Cd + S 2 (24.5 μg L^{-1}, + spirulina 4 mg L^{-1}), Cd + S 3 (24.5 μg L^{-1}, + spirulina 10 mg L^{-1}) for 96 h.

3. Results

3.1. FETAX assay

Exposure to cadmium (0.61, 2.45, 4.91, 9.82, 19.63, and 38.34 mg L^{-1}) mortality in the concentrations up to 4.91 mg L^{-1} was 100%, medium lethal concentration (LC_{50}) was 2.62 mg L^{-1} (*95% CI:0.724–3.160*), afterward lowest adverse effect level (LOAEL) was calculated to be 24.5 μg L^{-1}.

3.1.1. Mortality and malformations

The mortality of *X. laevis* exposed to cadmium and spirulina mixtures Cd + S 1, Cd + S 2, Cd + S 3 is shown in Fig. 1. Exposure to 10 mg L^{-1} of spirulina did not significantly differ with respect to the control group ($P < 0.05$), while reduction from 44.44% up to 50% was observed in spirulina mixtures, the most effective mixtures were Cd + S 2 and Cd + S 3. The number of larvae with malformations is shown in Fig. 2. The three spirulina mixtures (Cd + S 1, Cd + S 2, Cd + S 3) reduced malformations from 35.85% to 67.93%. The main malformations observed (Fig. 3) were: stunted embryos, gut miscoiling, rectum malformation, cardiac edema, abdominal edema, microcephaly, and axial malformations (bent tail, notochord, and fin). Also, spirulina mixtures (Cd + S 1, Cd + S 2, Cd + S 3) reduced the severity of malformations; Cd + S 3 was the most effective mixture in attenuating malformations, as shown in Fig. 4. To summarize, Cd + S 3 reduced mortality, the incidence of malformations, and the severity of malformations; it is also important to highlight that all spirulina mixtures reduced damage in some proportion.

3.1.2. Growth inhibition

Head-to-tail measurements of larvae are shown in Fig. 5. Embryos exposed to spirulina did not produce a significant difference with respect to the control group ($P < 0.05$). In contrast, spirulina mixtures reduced growth inhibition induced by cadmium, Cd + S 3 was the most effective mixture due to the length of larvae exposed to this mixture had a similar size than the control group according to statistical analysis ($P < 0.05$).

3.2. Oxidative stress evaluation

3.2.1. Lipid peroxidation

Fig. 6 shown lipid peroxidation levels, cadmium, and spirulina mixtures (Cd + S 1, Cd + S 2, Cd + S 3) increased lipid peroxidation levels compared to the control group; while the group exposed to spirulina (10 mg L^{-1}) did not present a significant difference with respect to the control group ($P < 0.05$). Nonetheless, all spirulina mixtures reduced lipid peroxidation levels compared to cadmium exposure. We observed that Cd + S 2 reduced lipid peroxidation levels effectively if compared to the other mixtures.

3.2.2. SOD activity

SOD activity is shown in Fig. 7; we observed an increase in SOD activity induced by cadmium exposure compared to control; however, the group exposed to spirulina (10 mg L^{-1}) presented the highest significant activity with respect to the control group ($P < 0.05$). While spirulina mixtures Cd + S 1, Cd + S 2, Cd + S 3 generated a decrease in SOD activity, a higher decrease was observed in Cd + S 2.

3.2.3. CAT activity

Fig. 8 shown catalase activity with a similar tendency as SOD, spirulina mixtures Cd + S 1, Cd + S 2, Cd + S 3 decreased CAT activity, but Cd + S 2 and Cd + S 3 shown the most effective reduction in CAT activity compared to cadmium, while the group exposed to spirulina (10 mg L^{-1}) has a significant difference with respect to the control group ($P < 0.05$), but the activity is minor than the mixtures.

4. Discussion

To determine if spirulina has a protective effect against the toxicity induced by cadmium in *Xenopus laevis*, we performed the FETAX assay. The most relevant effects evaluated in our study were mortality, macroscopic malformations, and growth inhibition. Spirulina (*Arthrospira maxima*) reduced mortality, incidence, and severity of malformations and enhance growth, and spirulina also decreases oxidative damage induced by cadmium 24.5 μg L^{-1} in *Xenopus laevis*.

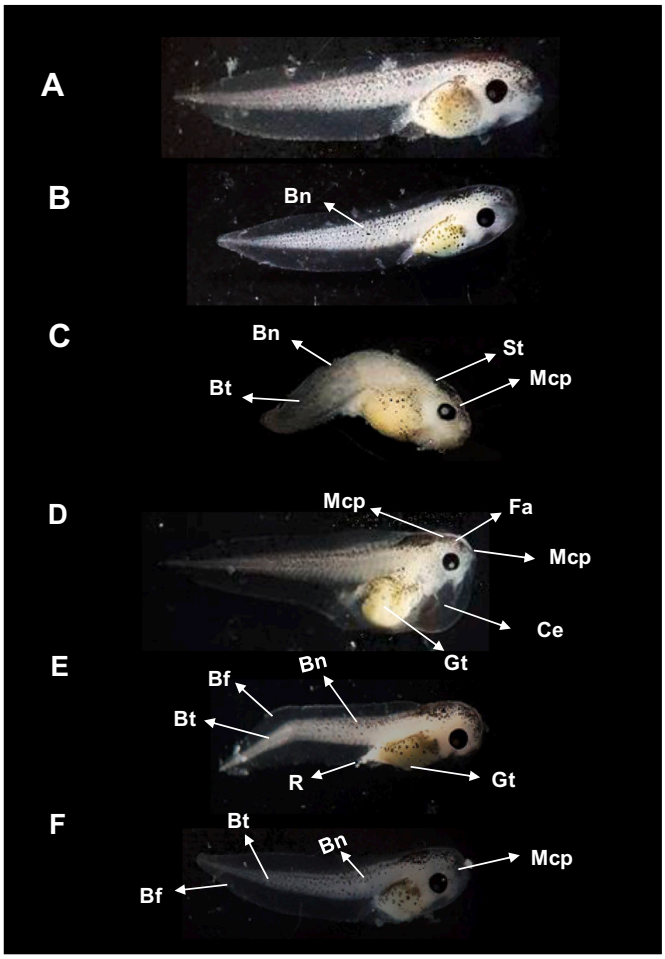

Fig. 4. Most frequent and representative malformations in *X. laevis* embryos after 96 h of exposure to (A) control, (B) spirulina 10 mg L^{-1}, (C) cadmium 24.5 μg L^{-1}, (D) Cd + S 1 (cadmium 24.5 μg L^{-1}+ spirulina 2 mg L^{-1}) (E) Cd + S 2 (cadmium 24.5 μg L^{-1} + spirulina 4 mg L^{-1}), (F) Cd + S 3 (cadmium 24.5 μg L^{-1} + spirulina 10 mg L^{-1}). Abbreviations: St: stunted, Gt: gut miscoiling, R: rectum, Mcp: microcephaly, Fa: face abnormality, Ea: eye abnormality, Bn: bent notochord, Bt: bent tail, Bf: bent fin, Ce: cardiac edema.

4.1. Mortality and malformations

The significant increase in mortality of *Xenopus laevis* larvae exposed to cadmium may be due to cadmium prooxidant activity, the alteration in nutrients absorption (Zn, Mg, and Cu), and disturbances of meiotic spindle morphogenesis (Slaby et al., 2017); this can lead to cell death; these effects were reported previously in *Xenopus laevis* exposed to cadmium (Sharma and Patiño, 2009). Groups exposed to Cd + S 1, Cd + S 2, Cd + S 3 had a reduction in mortality rate; a higher reduction was observed in Cd + S 3, with a decrease in mortality of 50%. The reduction in mortality may be due to the spirulina scavenge properties that generate protective effect against oxidative damage reducing ROS and NOS production; spirulina also has a chelating capacity to bind to heavy metals (Gelagutashvili, 2006), which can reduce the amount of cadmium-free to interact with biomolecules and reduce the disturbances in nutrient absorption and cellular damage.

Main malformations observed in cadmium exposure were: Stunted embryos, gut miscoiling, rectum malformation, cardiac edema,

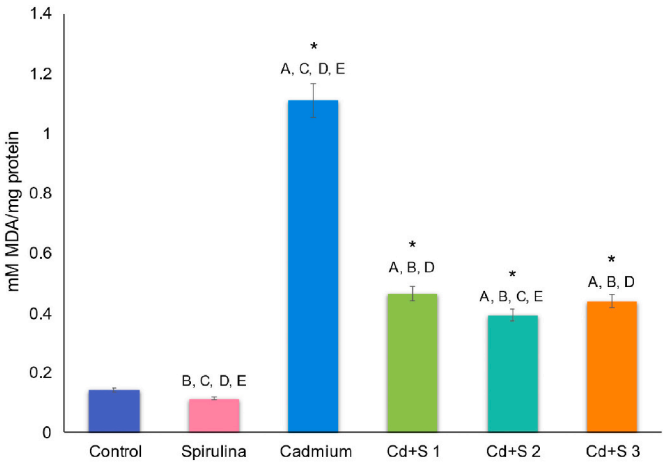

Fig. 6. LPX Lipid peroxidation in *Xenopus laevis* larvae exposed to: control, spirulina 10 mg L^{-1}, cadmium 24.5 μg L^{-1}, Cd + S 1 (cadmium 24.5 μg L^{-1} + spirulina 2 mg L^{-1}) Cd + S 2 (cadmium 24.5 μg L^{-1} + spirulina 4 mg L^{-1}), Cd + S 3 (cadmium 24.5 μg L^{-1} + spirulina 10 mg L^{-1}) for 192 h. Significant differences relative to: (*) control, (A) spirulina, (B) cadmium, (C) Cd + S 1, (D) Cd + S 2, (E) Cd + S 3. ($P < 0.05$).

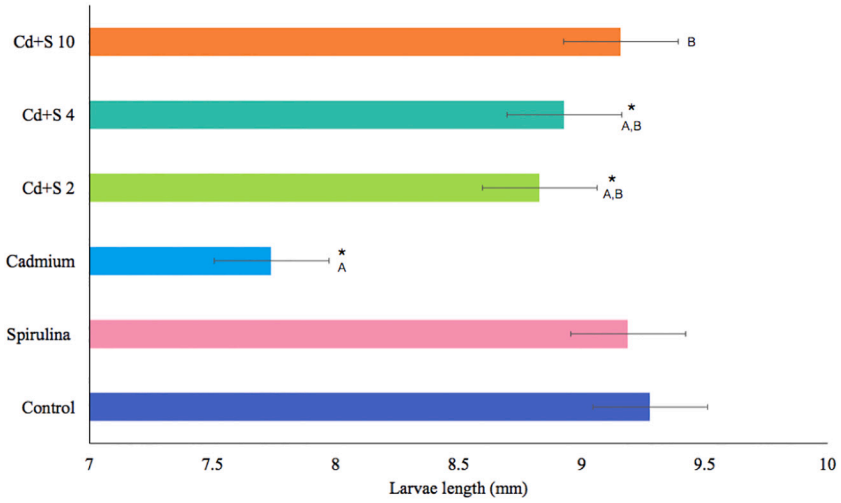

Fig. 5. Head to tail measurement of *Xenopus laevis* larvae exposed to control, spirulina 10 mg L^{-1}, cadmium 24.5 μg L^{-1}, Cd + S1 (cadmium 24.5 μg L^{-1} + spirulina 2 mg L^{-1}) Cd + S 2 (cadmium 24.5 μg L^{-1} + spirulina 4 mg L^{-1}), Cd + S3 (cadmium 24.5 μg L^{-1} + spirulina 10 mg L^{-1}) for 96 h, significant differences relative to: (*) control (A) spirulina, (B) cadmium ($P < 0.05$).

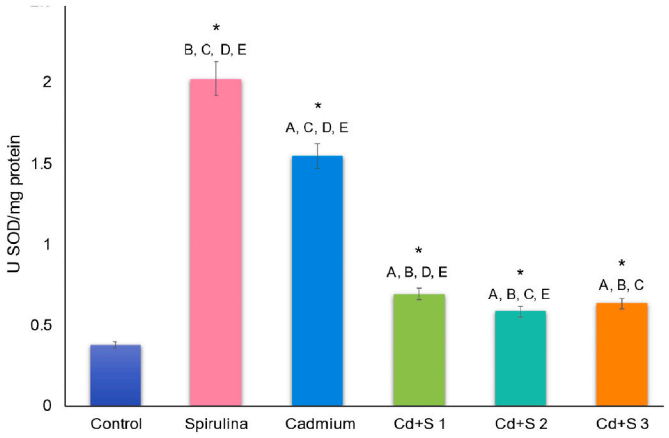

Fig. 7. SOD Superoxide dismutase activity in *Xenopus laevis* larvae exposed to control, spirulina 10 mg L^{-1}, cadmium 24.5 μg L^{-1}, Cd + S 1 (cadmium 24.5 μg L^{-1} + spirulina 2 mg L^{-1}) Cd + S 2 (cadmium 24.5 μg L^{-1} + spirulina 4 mg L^{-1}), Cd + S 3 (cadmium 24.5 μg L^{-1} + spirulina 10 mg L^{-1}) for 192 h. Significant differences relative to: (*) control, (A) spirulina, (B) cadmium, (C) Cd + S 1, (D) Cd + S 2, (E) Cd + S 3. ($P < 0.05$).

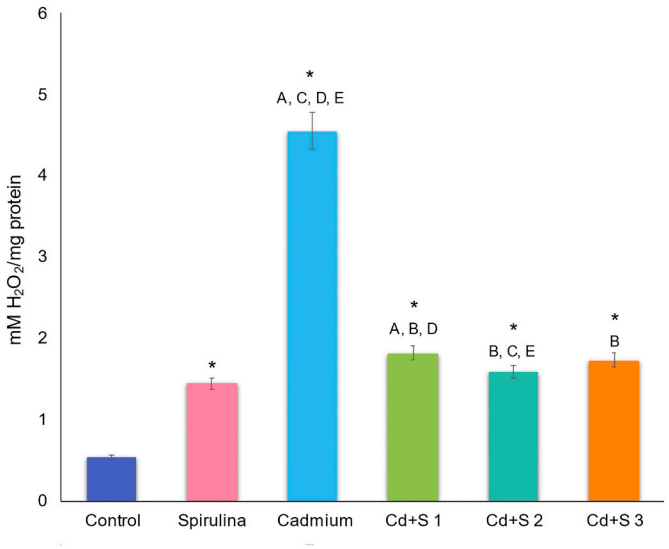

Fig. 8. CAT Catalase activity in *Xenopus laevis* larvae exposed to control, cadmium 24.5 μg L^{-1}, spirulina 10 mg L^{-1}, Cd + S 1 (cadmium 24.5 μg L^{-1} + spirulina 2 mg L^{-1}) Cd + S 2 (cadmium 24.5 μg L^{-1} + spirulina 4 mg L^{-1}), Cd + S 3 (cadmium 24.5 μg L^{-1} + spirulina 10 mg L^{-1}) for 192 h. Significant differences relative to: (*) control, (A) spirulina, (B) cadmium, (C) Cd + S 1, (D) Cd + S 2, (E) Cd + S 3. ($P < 0.05$).

abdominal edema, microcephaly, and axial malformations (bent tail, notochord, and fin); similar to those previously reported in *Xenopus laevis* (Sunderman et al., 1991) *Bufo gargarizans* (Wu et al., 2017b), *Danio rerio* (Cheng et al., 2000) and *Silurus soldatovi* (Zhang et al., 2012). Cadmium modifies apoptosis, cell cycle, stress, and immune response (Liu et al., 2018), induce p53 phosphorylation, can replace Zn in the zinc-finger domain, and cause errors in DNA repair resulting in the accumulation of damaged DNA (Chen and Shaikh, 2009; Dally and Hartwig, 1997) and affect signaling processes, generates oxidative stress and potential redox alterations. As organogenesis is the most vulnerable stage of development and requires signaling processes to regulate proliferation and cell differentiation (Hayashi et al., 2018); cadmium can cause failure on embryonic development, thus generate changes in tissues function and structure and, as a consequence, diverse malformations (Laforgia et al., 2018). Spirulina mixtures Cd + S 1, Cd + S 2, Cd + S 3 generated a decrease in incidence and severity of malformations in larvae exposed to cadmium. Argüelles-Velázquez et al. (2013) also found a reduction in the frequency of malformations with the supplementation of spirulina in rats exposed to cadmium; this may be due to the effect of phycobiliproteins; which have anti-inflammatory activity (Khafaga and El-Sayed, 2018) by reducing inflammation, edema can be reduced, due to most of the edema is produced by a chronic inflammatory process. Phycocyanin and β-carotenes inhibit the formation of proinflammatory cytokines, thus suppressing the expression of cyclooxygenase II and the production of prostaglandin E2, which acts as an inflammatory mediator. Phycocyanin has antioxidant effects, can eliminate hydroxyl radicals responsible for oxidative damage (Bermejo et al., 2008), and reduce oxidative stress. The reduction in cellular damage can reduce the severity and incidence of malformations in *Xenopus laevis*.

4.2. Growth inhibition

Cadmium exposure decreased larvae length; this effect was also observed in previous studies. Wu et al. (2017b) reported a reduction in total length induced by Cd in *Buffo Garganzianis*, which may be due to can mimic other essential minerals, thus inhibits nutrient absorption (Goyer, 1995), and trigger failures in developmental processes, and generate alterations in calcium-dependent processes, which are highly important in early life stages (Borodinsky, 2017; Hayashi et al., 2018).

The exposure to spirulina mixtures (Cd + S 1, Cd + S 2, Cd + S 3) shown beneficial effects in development, total body length increased compared to cadmium exposure, in mixture Cd + S 3 larvae had a similar size to larvae from the control group. This may be due to the spirulina cell wall is porous and allows cadmium a free pass-thru; when cadmium gets to the intracellular compartment, chelating agents act binding to cadmium and neutralizing it (Bermejo et al., 2008); these chelating agents are often induced by heavy metals exposure (Knauer et al., 1998). Another essential mediator is phycocyanin which can scavenge hydroxyl, alkoxy, and peroxyl radicals that may initiate the arachidonic acid cascade; phycocyanin also blocks the phosphorylation of p38 mitogen-active protein kinases, which regulate the synthesis of cytokines, including TNF- α and ILe1β (Khalil et al., 2017). Vitamins, proteins, and minerals of spirulina may also be involved in the enhance of development. Similar results have been reported previously in rabbits and rats exposed to lead (Pb) and spirulina mixtures. The total size and weight of the organisms were diminished by Pb exposure, and after spirulina supplementation, weight and size increased significantly (Aladaileh et al., 2020).

4.3. Oxidative stress

According to Ercal et al. (2001), the mechanisms responsible for Cd-induced toxicity may be multifactorial; it can induce adverse effects on cellular defense systems and thiol status, enhance lipid peroxidation, and causes deleterious effects on cellular enzymes.

Cadmium can generate alterations in mitochondrial processes, the primary source of ROS, such as electron transfer chain and energy generation (ATP), as mitochondria is a suitable source of oxygen when cadmium alters the activity of complexes II (succinate: ubiquinone oxidoreductase) and III (ubiquinol: cytochrome C oxidoreductase), lead to an excessive ROS generation and hence oxidative damage (Gobe and Crane, 2010; Wang et al., 2004).

Cadmium exposure induced adverse effects in aquatic organisms previously (Avallone et al., 2015; Cambier et al., 2010; Chan and Cheng, 2003; Mouchet et al., 2006; Pizzi et al., 2017; Shirriff and Heikkila, 2017; Slaby et al., 2016; Thompson and Bannigan, 2008; Wu et al., 2017b, 2017a; Xia et al., 2016) and many of this are related to oxidative stress. The ASTM guideline (ASTM E1439 - 12, 2019) recommends using concentrations lower than 10 μg L^{-1} to evaluate Cd-induced teratogenesis. However, there are reports in which higher concentrations are used to observe the toxic effect regarding the evaluation of cadmium-

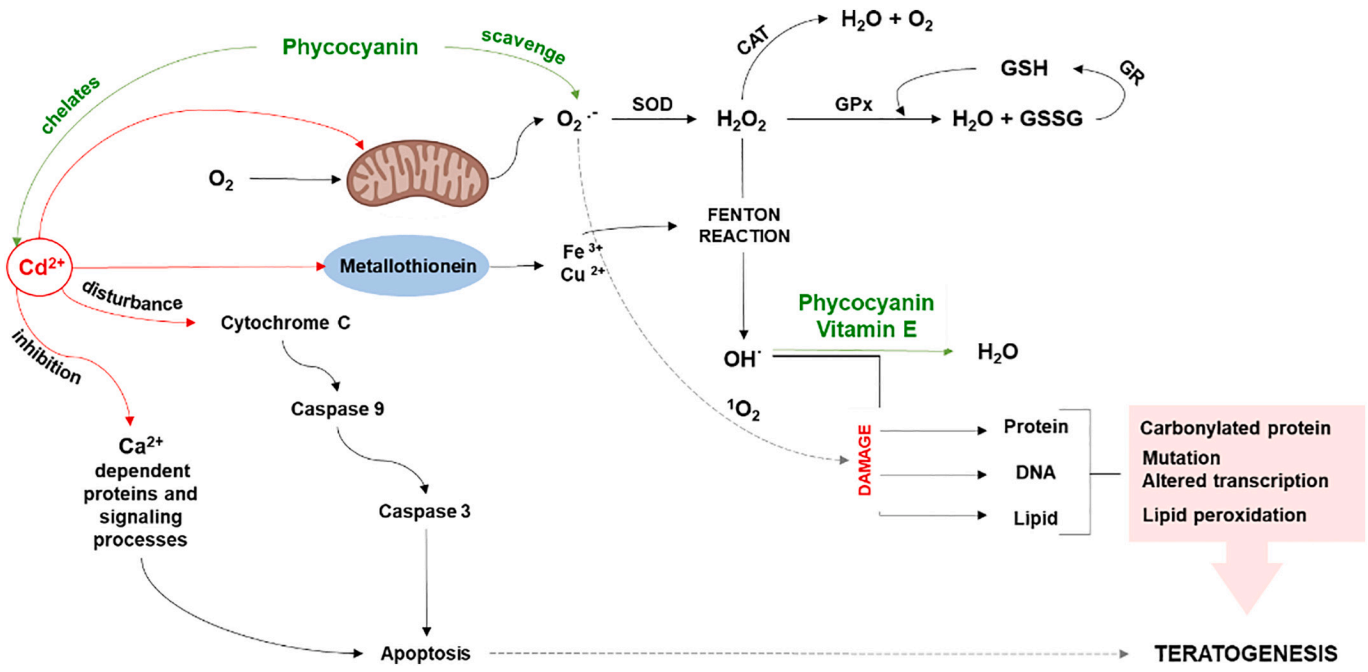

Fig. 9. Proposed route through oxidative damage is minimized by spirulina due to the chelation of cadmium and scavenge of ROS and RNS induced in *Xenopus laevis* exposed to cadmium.

induced oxidative stress. For example, a study in *Bufo gargarizans* was performed at nominal concentrations of cadmium 5, 50, 100, 200, and 500 μg L^{-1} (Wu et al., 2017a); in *Silurus soldatovi*, they tested nominal concentrations of Cd in a range from 1 to 16,000 μg L^{-1} to evaluate the toxicity on embryos and pro larva organisms (Zhang et al., 2012); these concentrations are similar to the concentrations tested in our work considering the early life stages of *X. laevis*; also, other studies use higher concentrations in adult organisms, as reported by Kasherwani et al. (2009) in Freshwater Catfish, *Heteropneustes fossilis*, who tested cadmium chloride at concentrations of 180, 240,320, 420, 560 mg L^{-1}. Our results showed that exposure of 24.5 μg L^{-1} of $CdCl_2$ induced an increase in Malondialdehyde (MDA), a well-known lipid peroxidation indicator. This may be attributed to excessive ROS production, which interacted with the cell membrane and triggered the formation of lipid radicals, causing a loss of membrane function and hence cellular damage; specifically, it has been suggested that disturbances in GSH and metallothionein levels may allow free radicals as $HO^{\bullet}$ and $O2^{\cdot -}$ radicals, this can attack double bonds in membrane lipids and increase lipid peroxidation. On the other hand, mitochondrial respiration is promoted by lipid peroxidation and enhances oxidative stress (Karmakar et al., 1998). Nonetheless, spirulina mixtures exposure (Cd + S 1, Cd + S 2, Cd + S3) achieved a reduction in lipid peroxidation levels, Cd + S 2 shown a reduction statistically significant compared to Cd + S 1 and Cd + S3; similar effects of a decrease in lipid peroxidation with spirulina supplementation have been reported previously in *Oreochromis niloticus* exposed to deltamethrin, (Abdelkhalek et al., 2017); in rats exposed to sodium fluoride (Banji et al., 2013), Wistar rats exposed to methotrexate (Khafaga and El-Sayed, 2018), *Clarias gariepinus* exposed to sodium dodecyl sulfate (Sayed and Authman, 2018), rabbits exposed to lead acetate (Aladaileh et al., 2019), male rats exposed to sodium arsenite (Bashandy et al., 2016) and Wistar rats exposed to Cadmium (Karadeniz et al., 2009). As mentioned previously, spirulina has the chelating ability to bind cadmium ions and inhibit Fenton reaction; it also can neutralize alkoxyl hydroxyl and peroxyl radicals (Wu et al., 2016), and if the lipid peroxidation process is inhibited in an early stage, damage can be reduced. Vitamin E in spirulina can be protective against lipid peroxidation due it has a chroman ring on its structure that apports reductive effect and can reduce peroxyl radicals into hydroperoxides which afterward can be degraded enzymatically (Miyazawa et al., 2019). Carotenoids in spirulina also have antioxidant activity, scavenge ROS, and neutralize oxygen singlet (Stahl and Sies, 2003).

The antioxidant enzymatic activity was also increased in cadmium exposure; it has been reported previously that especially CAT and SOD, two major antioxidant enzymes, are affected by Cd and can induce an increase in enzymatic activity (Ercal et al., 2001; Karadeniz et al., 2009; Wu et al., 2017a; Xia et al., 2016). As mentioned before, Cd has a multifactorial mechanism of toxicity, none the less; one way for the production of reactive oxygen species is through its entrance to the electron transport chain in mitochondria, leading to the accumulation of unstable semi ubiquinones which donate electrons and create superoxide radicals (Prabhat et al., 2019). The increase in enzymatic activity can result from excessive production of reactive species; cadmium generates ROS and RNS and increases superoxide ($O_2^{\cdot -}$) levels. Then, SOD catalyzes a dismutation process, transforming $O_2^{\cdot -}$ into H_2O_2, which CAT and glutathione peroxidase later transform into H_2O (Liu et al., 2018); these processes induce an increase in antioxidant enzyme activity to neutralize ROS and protect the cell from oxidative damage. As shown in Fig. 7, the increment in superoxide radicals induced by Cd increases the level of SOD, which is later decreased by spirulina treatment in the mixtures. However, exposure to spirulina (10 mg L^{-1}) generated the highest increase in the activity of this enzyme, which has been reported in previous studies. It may be due to spirulina have SOD on its composition (Asieh et al., 2016); while, groups exposed to spirulina mixtures Cd + S 1, Cd + S 2, and Cd + S3 shown a decrease in both enzymatic activity, the higher decrease statistically significant ($P < 0.05$) in SOD activity was induced by Cd + S 2, whereas the higher decrease statistically significant ($P < 0.05$) in CAT activity was induced by Cd + S 3.

Regarding catalase activity, similar results were observed by Amr et al. (2006), who reported that catalase activity was increased by cadmium and further decreased in rats treated with spirulina. As mentioned above, spirulina can improve antioxidant activity and help to restore the ability to neutralize ROS and RNS, therefore reduce oxidative damage (Fig. 9). Other studies where supplementation with spirulina was performed report an increase in antioxidant enzymes (Abdelkhalek et al., 2017; Banji et al., 2013; Karadeniz et al., 2009; Sayed and Authman, 2018); contrary to the results obtained in this work, this may

be due other studies are focused on an adult organism, or in embryos in the gestational process (mammals), and in early life stages some recovery responses are not fully developed, this may contribute to a different response against damage, in addition, it depends on the toxicity mechanism of the metal that has been evaluated.

5. Conclusions

Cadmium can generate diverse alterations, induce an increase in ROS and RNS, inhibit nutrients absorption, and generate disturbances in some signaling processes, hence cellular damage and malformations. On the other hand, spirulina can scavenge highly toxic radicals such as hydroxyl, peroxyl, and superoxide radical, also spirulina can chelate cadmium, inhibiting damage in cellular components, reducing malformations, growth inhibition, and oxidative damage. Spirulina (*Arthrospira maxima*) shown protective effects against cadmium-induced toxicity in *Xenopus laevis*, the higher beneficial effects were observed in 4 and 10 mg L^{-1}; nonetheless, 2 mg L^{-1} also shown significant positive effects: oxidative damage was reduced, mortality and malformation rate decrease, as well as the severity of malformations and growth inhibition. Spirulina can be considered as a diet complement for amphibians to prevent toxicity induced by metals in early life stages, further studies focused on the effects of spirulina in amphibians and aquatic organisms are recommended.

Declaration of competing interest

All of the authors have read and approved the manuscript and declare that they have no conflicts of interest to declare that they are relevant to the content of this article.

References

Abdelkhalek, N.K.M., Ghazy, E.W., 2014. Pharmacodynamic interaction of Spirulina platensis and deltamethrin in freshwater fish Nile tilapia, Oreochromis niloticus: impact on lipid peroxidation and oxidative stress. https://doi.org/10.1007/s11356-014-3578-0.

Abdelkhalek, N.K.M., Eissa, I.A.M., Ahmed, E., Kilany, O.E., El-Adl, M., Dawood, M.A.O., Hassan, A.M., Abdel-Daim, M.M., 2017. Protective role of dietary Spirulina platensis against diazinon-induced oxidative damage in Nile tilapia; Oreochromis niloticus. Environ. Toxicol. Pharmacol. 54 https://doi.org/10.1016/j.etap.2017.07.002.

Adeyemi, J.A., Da Cunha Martins, A., Barbosa, F., 2015. Teratogenicity, genotoxicity and oxidative stress in zebrafish embryos (Danio rerio) co-exposed to arsenic and atrazine. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 172–173, 7–12. https://doi.org/10.1016/j.cbpc.2015.04.001.

Aladaileh, S.H., Khafaga, A.F., El-hack, M.E.A., Al-gabri, N.A., 2019. Spirulina platensis ameliorates the sub chronic toxicities of lead in rabbits via anti-oxidative, anti-inflammatory, and immune stimulatory properties. Sci. Total Environ. 701, 134879 https://doi.org/10.1016/j.scitotenv.2019.134879.

Aladaileh, S.H., Khafaga, A.F., Abd El-Hack, M.E., Al-Gabri, N.A., Abukhalil, M.H., Alfwuaires, M.A., Bin-Jumah, M., Alkahtani, S., Abdel-Daim, M.M., Aleya, L., Abdelnour, S., 2020. Spirulina platensis ameliorates the sub chronic toxicities of lead in rabbits via anti-oxidative, anti- inflammatory, and immune stimulatory properties. Sci. Total Environ. 701, 134879 https://doi.org/10.1016/j.scitotenv.2019.134879.

Al-homaidan, A.A., Alabdullatif, J.A., Al-hazzani, A.A., Al-ghanayem, A.A., Alabbad, A. F., 2015. Adsorptive removal of cadmium ions by Spirulina platensis dry biomass. Saudi J. Biol. Sci. 6–11. https://doi.org/10.1016/j.sjbs.2015.06.010.

Amr, Hamza, Alaaeldin A., Daoud, Sayel, Hamza, W., 2006. Spirulina protects against cadmium-induced hepatotoxicity in rats. Am. J. Pharmacol. Toxicol. 1 (2), 5. https://doi.org/10.3844/ajptsp.2006.21.25.

Argüelles-Velázquez, N., Alvarez-González, I., Madrigal-Bujaidar, E., Chamorro-Cevallos, G., 2013. Amelioration of cadmium-produced teratogenicity and genotoxicity in mice given Arthrospira maxima (Spirulina) treatment, 2013. https://doi.org/10.1155/2013/604535.

Asieh, A., Fazilati, M., Mohammad Latifi, A., Salavati, H., Choopani, A., 2016. A review on antioxidant properties of spirulina. J. Appl. Biotechnol. Rep. 3 (1), 345–351.

ASTM (American Society for Testing Materials), 2019. Standard Practice for Conducting an Interlaboratory Study to Determine the Precision of a Test Method, 1, pp. 1–23. https://doi.org/10.1520/E1439-12R19.

Authman, M.M., 2015. Use of fish as bio-indicator of the effects of heavy metals pollution. J. Aquac. Res. Dev. 06 https://doi.org/10.4172/2155-9546.1000328.

Avallone, B., Agnisola, C., Cerciello, R., Panzuto, R., 2015. Structural and functional changes in the zebrafish (Danio rerio) skeletal muscle after cadmium exposure. Cell Biol. Toxicol. https://doi.org/10.1007/s10565-015-9310-0.

Banji, D., Banji, O.J.F., Pratusha, N.G., Annamalai, A.R., 2013. Investigation on the role of Spirulina platensis in ameliorating behavioural changes, thyroid dysfunction and oxidative stress in offspring of pregnant rats exposed to fluoride. Food Chem. 140, 321–331. https://doi.org/10.1016/j.foodchem.2013.02.076.

Bantle, J.A., Dumont, J.N., Finch, R., Linder, G., 1991. Atlas of Abnormalities: A Guide for the Performance of FETAX. OKlahoma State University Publications Departament, Still- water, OK.

Bashandy, S.A.E., El Awdan, S.A., Ebaid, H., Alhazza, I.M., 2016. Antioxidant potential of Spirulina platensis mitigates oxidative stress and reprotoxicity induced by sodium arsenite in male rats, 2016.

Bermejo, P., Piñero, E., Villar, Á.M., 2008. Iron-chelating ability and antioxidant properties of phycocyanin isolated from a protean extract of Spirulina platensis. Food Chem. 110, 436–445. https://doi.org/10.1016/j.foodchem.2008.02.021.

Boehringer, M.-M., 1987. Biochemical Information. A Revised Biochemical Reference Sources. Enzymes for Routine, 1st ed. (Germany).

Bonfanti, P., Colombo, A., Orsi, F., Nizzetto, I., Andrioletti, M., Bacchetta, R., Mantecca, P., Fascio, U., Vailati, G., Vismara, C., 2004. Comparative teratogenicity of Chlorpyrifos and Malathion on Xenopus laevis development. Aquat. Toxicol. 70, 189–200. https://doi.org/10.1016/j.aquatox.2004.09.007.

Borodinsky, L.N., 2017. *Xenopus laevis* as a model organism for the study of spinal cord formation, development, function and regeneration, 11, pp. 1–9. https://doi.org/10.3389/fncir.2017.00090.

Brausch, J.M., Wages, M., Shannahan, R.D., Perry, G., Anderson, T.A., Maul, J.D., Mulhearn, B., Smith, P.N., 2010. Surface water mitigates the anti-metamorphic effects of perchlorate in New Mexico spadefoot toads (Spea multiplicata) and African clawed frogs (Xenopus laevis). Chemosphere 78, 280–285. https://doi.org/10.1016/j.chemosphere.2009.10.049.

Buege, J.A., Aust, S.D., 1978. Microsomal lipid peroxidation. Methods Enzymol. 52, 302–310.

Cambier, S., Gonzalez, P., Durrieu, G., Bourdineaud, J.P., 2010. Cadmium-induced genotoxicity in zebrafish at environmentally relevant doses. Ecotoxicol. Environ. Saf. 73, 312–319. https://doi.org/10.1016/j.ecoenv.2009.10.012.

Cardoso-Vera, J.D., Islas-Flores, H., SanJuan-Reyes, N., Montero-Castro, E.I., Galar-Martínez, M., García-Medina, S., Elizalde-Velázquez, A., Dublán-García, O., Gómez-Oliván, L.M., 2017. Comparative study of diclofenac-induced embryotoxicity and teratogenesis in Xenopus laevis and Lithobates catesbeianus, using the frog embryo teratogenesis assay: Xenopus (FETAX). Sci. Total Environ. 574, 467–475. https://doi.org/10.1016/j.scitotenv.2016.09.095.

Castro-García, S.Z., Chamorro-Cevallos, G., Quevedo-Corona, L., McCarty, M.F., Bobadilla-Lugo, R.A., 2018. Beneficial effects of phycobiliproteins from Spirulina maxima in a preeclampsia model. Life Sci. 211, 17–24. https://doi.org/10.1016/j.lfs.2018.09.011.

Chan, P.K., Cheng, S.H., 2003. Cadmium-induced ectopic apoptosis in zebrafish embryos. Arch. Toxicol. 77, 69–79. https://doi.org/10.1007/s00204-002-0411-1.

Chen, J., Shaikh, Z.A., 2009. Activation of Nrf2 by cadmium and its role in protection against cadmium-induced apoptosis in rat kidney cells. Toxicol. Appl. Pharmacol. 241, 81–89. https://doi.org/10.1016/j.taap.2009.07.038.

Cheng, S.H., Wai, A.W.K., So, C.H., Wu, R.S.S., 2000. Cellular and molecular basis of cadmium-induced deformities in zebrafish embryos. Environ. Toxicol. Chem. 19, 3024–3031. https://doi.org/10.1002/etc.5620191223.

Dally, H., Hartwig, A., 1997. Induction and repair inhibition of oxidative DNA damage by nickel(II) and cadmium(II) in mammalian cells. Carcinogenesis 18, 1021–1026. https://doi.org/10.1093/carcin/18.5.1021.

de la Jara, A., Ruano-Rodriguez, C., Polifrone, M., Assunçao, P., Brito-Casillas, Y., Wägner, A.M., Serra-Majem, L., 2018. Impact of dietary Arthrospira (Spirulina) biomass consumption on human health: main health targets and systematic review. J. Appl. Phycol. 30, 2403–2423. https://doi.org/10.1007/s10811-018-1468-4.

Deng, R., Chow, T.-J., 2010. Hypolipidemic, antioxidant, and antiinflammatory activities of microalgae Spirulina. Cardiovasc. Ther. 28, e33–e45. https://doi.org/10.1111/j.1755-5922.2010.00200.x.

Dennery, P.A., 2007. Effects of oxidative stress on embryonic development. Birth Defects Res. C Embryo Today 81, 155–162. https://doi.org/10.1002/bdrc.20098.

Ercal, N., Gurer-Orhan, H., Aykin-Burns, N., 2001. Toxic metals and oxidative stress part I: mechanisms involved in metal induced oxidative damage. Curr. Top. Med. Chem. 1, 529–539. https://doi.org/10.2174/1568026013394831.

Farag, M.R., El-aziz, R.M.A., Ali, H.A., Ahmed, S.A., 2015. Evaluating the ameliorative efficacy of Spirulina platensis on spermatogenesis and steroidogenesis in cadmium-intoxicated rats. https://doi.org/10.1007/s11356-015-5314-9.

Ferruzzi, M.G., Blakeslee, J., 2007. Digestion, absorption, and cancer preventative activity of dietary chlorophyll derivatives. Nutr. Res. 27, 1–12. https://doi.org/10.1016/j.nutres.2006.12.003.

Ganesan, S., Anaimalai Thirumurthi, N., Raghunath, A., Vijayakumar, S., Perumal, E., 2016. Acute and sub-lethal exposure to copper oxide nanoparticles causes oxidative stress and teratogenicity in zebrafish embryos. J. Appl. Toxicol. 36, 554–567. https://doi.org/10.1002/jat.3224.

Gelagutashvili, E., 2006. Interaction of Cu(II) and Cd(II) ions with DNA from Spirulina platensis. J. Therm. Anal. Calorim. 85, 491–494. https://doi.org/10.1007/s10973-006-7696-x.

Giaginis, C., Gatzidou, E., Theocharis, S., 2006. DNA repair systems as targets of cadmium toxicity. Toxicol. Appl. Pharmacol. 213, 282–290. https://doi.org/10.1016/j.taap.2006.03.008.

Gilbert-Barness, E., 2010. Teratogenic causes of malformations. Ann. Clin. Lab. Sci. 40, 99–114.

Gobe, G., Crane, D., 2010. Mitochondria, reactive oxygen species and cadmium toxicity in the kidney. Toxicol. Lett. 198, 49–55. https://doi.org/10.1016/j.toxlet.2010.04.013.

Goyer, R.A., 1995. Nutrition and metal toxicity. Am. J. Clin. Nutr. 61, 646S–650S. https://doi.org/10.1093/ajcn/61.3.646S.
Hayashi, K., Yamamoto, T.S., Ueno, N., 2018. Intracellular calcium signal at the leading edge regulates mesodermal sheet migration during Xenopus gastrulation, pp. 1–12. https://doi.org/10.1038/s41598-018-20747-w.
Hayati, A., Pratiwi, H., Khoiriyah, I., Winarni, D., Sugiharto, 2017. Histopathological assessment of cadmium effect on testicles and kidney of Oreochromis niloticus in different salinity. AIP Conf. Proc. 1854 https://doi.org/10.1063/1.4985405.
Hopkins, W.A., 2007. Amphibians as models for studying environmental change. ILAR J. 48, 270–277. https://doi.org/10.1093/ilar.48.3.270.
Hu, L., Zhu, J., Rotchell, J.M., Wu, L., Gao, J., Shi, H., 2015. Use of the enhanced frog embryo teratogenesis assay- Xenopus (FETAX) to determine chemically-induced phenotypic effects. Sci. Total Environ. 508, 258–265. https://doi.org/10.1016/j.scitotenv.2014.11.086.
Ishibashi, Shoko, Amaya, Enrique, 2020. How to grow *Xenopus laevis* tadpole stages to adult. Cold Spring Harb. Protoc. https://doi.org/10.1101/pdb.prot106245.
Isidori, M., Piscitelli, C., Russo, C., Smutná, M., Bláha, L., 2016. Teratogenic effects of five anticancer drugs on Xenopus laevis embryos. Ecotoxicol. Environ. Saf. 133, 90–96. https://doi.org/10.1016/j.ecoenv.2016.06.044.
Järup, L., Åkesson, A., 2009. Current status of cadmium as an environmental health problem. Toxicol. Appl. Pharmacol. 238, 201–208. https://doi.org/10.1016/j.taap.2009.04.020.
Joventino, I.P., Alves, H.G.R., Neves, L.C., Pinheiro-Joventino, F., Leal, L.K.A.M., Neves, S.A., Ferreira, F.V., Brito, G.A.C., Viana, G.B., 2012. The microalga Spirulina platensis presents anti-inflammatory action as well as hypoglycemic and hypolipidemic properties in diabetic rats. J. Complement. Integr. Med. 9 https://doi.org/10.1515/1553-3840.1534.
Karadeniz, A., Cemek, M., Simsek, N., 2009. The effects of *Panax ginseng* and Spirulina platensis on hepatotoxicity induced by cadmium in rats. Ecotoxicol. Environ. Saf. 72, 231–235. https://doi.org/10.1016/j.ecoenv.2008.02.021.
Karmakar, R., Banik, S., Bandyopadhyay, S., Chatterjee, M., 1998. Cadmium-induced alterations of hepatic lipid peroxidation, glutathione S-transferase activity and reduced glutathione level and their possible correlation with chromosomal aberration in mice: a time course study. Mutat. Res. 397, 183–190. https://doi.org/10.1016/s0027-5107(97)00209-1.
Kasherwani, D., Lodhi, H.S., Tiwari, K.J., Shukla, S., Sharma, U., 2009. Cadmium toxicity to freshwater catfish, *Heteropneustes fossilis* (Bloch). Asian J. Exp. Sci. 23, 149–156.
Khafaga, A.F., El-Sayed, Y.S., 2018. Spirulina ameliorates methotrexate hepatotoxicity via antioxidant, immune stimulation, and proinflammatory cytokines and apoptotic proteins modulation. Life Sci. 196, 9–17. https://doi.org/10.1016/j.lfs.2018.01.010.
Khalil, S.R., Reda, R.M., Awad, A., 2017. Efficacy of Spirulina platensis diet supplements on disease resistance and immune-related gene expression in Cyprinus carpio L. exposed to herbicide atrazine. Fish Shellfish Immunol. 67, 119–128. https://doi.org/10.1016/j.fsi.2017.05.065.
Knauer, K., Ahner, B., Xue, H. Bin, Sigg, L., 1998. Metal and phytochelatin content in phytoplankton from freshwater lakes with different metal concentrations. Environ. Toxicol. Chem. 17, 2444–2452. https://doi.org/10.1002/etc.5620171210.
Kulshreshtha, A., J., A., Jarouliya, U., Bhadauriya, P., Prasad, G., Bisen, P., 2008. Spirulina in health care management. Curr. Pharm. Biotechnol. 9, 400–405. https://doi.org/10.2174/138920108785915111.
Kumar, P., Desai, N., Dwivedi, M., 2016. Multiple potential roles of Spirulina in human health: a critical review. Malays. J. Nutr. 21, 375–387.
Laforgia, N., Di Mauro, A., Favia Guarnieri, G., Varvara, D., De Cosmo, L., Panza, R., Capozza, M., Baldassarre, M.E., Resta, N., 2018. The role of oxidative stress in the pathomechanism of congenital malformations. Oxidative Med. Cell. Longev. 2018, 1–12. https://doi.org/10.1155/2018/7404082.
Lima, F.A.V., Joventino, I.P., Joventino, F.P., de Almeida, A.C., Neves, K.R.T., do Carmo, M.R., Leal, L.K.A.M., de Andrade, G.M., de Barros Viana, G.S., 2017. Neuroprotective activities of Spirulina platensis in the 6-OHDA model of Parkinson's disease are related to its anti-inflammatory effects. Neurochem. Res. 42, 3390–3400. https://doi.org/10.1007/s11064-017-2379-5.
Liu, S., Yang, R., Tripathi, D.K., Li, X., He, W., Wu, M., Ali, S., Ma, M., Cheng, Q., Pan, Y., 2018. The interplay between reactive oxygen and nitrogen species contributes in the regulatory mechanism of the nitro-oxidative stress induced by cadmium in Arabidopsis. J. Hazard. Mater. 344, 1007–1024. https://doi.org/10.1016/j.jhazmat.2017.12.004.
Mahmoud, M.M.A., El-Lamie, M.M.M., Kilany, O.E., Dessouki, A.A., 2018. Spirulina (Arthrospira platensis) supplementation improves growth performance, feed utilization, immune response, and relieves oxidative stress in Nile tilapia (Oreochromis niloticus) challenged with Pseudomonas fluorescens. Fish Shellfish Immunol. 72, 291–300. https://doi.org/10.1016/j.fsi.2017.11.006.
Marin, M., Slaby, S., Marchand, G., Demuynck, S., Friscourt, N., Gelaude, A., Lemière, S., Bodart, J.F., 2015. Xenopus laevis oocyte maturation is affected by metal chlorides. Toxicol. In Vitro 29, 1124–1131. https://doi.org/10.1016/j.tiv.2015.04.016.
Miyazawa, Taiki, Burdeos, G.C., Itaya, M., Nakagawa, K., Miyazawa, Teruo, 2019. Vitamin E: regulatory redox interactions. IUBMB Life 71, 430–441. https://doi.org/10.1002/iub.2008.
Mouchet, F., Baudrimont, M., Gonzalez, P., Cuenot, Y., Bourdineaud, J.P., Boudou, A., Gauthier, L., 2006. Genotoxic and stress inductive potential of cadmium in Xenopus laevis larvae. Aquat. Toxicol. 78, 157–166. https://doi.org/10.1016/j.aquatox.2006.02.029.
Mühling, M., Belay, A., Whitton, B.A., 2005. Variation in fatty acid composition of Arthrospira (Spirulina) strains. J. Appl. Phycol. 17, 137–146. https://doi.org/10.1007/s10811-005-7213-9.
Nasirian, F., Dadkhah, M., Moradi-Kor, N., Obeidavi, Z., 2018. Effects of Spirulina platensis microalgae on antioxidant and anti-inflammatory factors in diabetic rats. Diabetes Metab. Syndr. Obes. Targets Ther. 11, 375–380. https://doi.org/10.2147/DMSO.S172104.
Nieuwkoop, P.D., Faber, J., 1994. Normal Table of *Xenopus laevis* (Daudin). A Systematical and Chronological Survey of the Development From the Fertilized Egg Till the End of Metamorphosis. Garland Publishing, Inc, New York.
Pašková, V., Hilscherová, K., Bláha, L., 2011. Mechanisms of hexavalent chromium resistance and removal by microorganisms. Rev. Environ. Contam. Toxicol. 211 https://doi.org/10.1007/978-1-4419-8011-3.
Pérez-Alvarez, I., Islas-Flores, H., Gómez-Oliván, L.M., Barceló, D., López De Alda, M., Pérez Solsona, S., Sánchez-Aceves, L., SanJuan-Reyes, N., Galar-Martínez, M., 2018. Determination of metals and pharmaceutical compounds released in hospital wastewater from Toluca, Mexico, and evaluation of their toxic impact. Environ. Pollut. 240, 330–341. https://doi.org/10.1016/j.envpol.2018.04.116.
Pérez-Juárez, A., Chamorro, G., Alva-Sánchez, C., Paniagua-Castro, N., Pacheco-Rosado, J., 2016. Neuroprotective effect of Arthrospira (Spirulina) platensis against kainic acid-neuronal death. Pharm. Biol. 54, 1408–1412. https://doi.org/10.3109/13880209.2015.1103756.
Peuchen, E.H., Sun, L., Dovichi, N.J., 2016. Optimization and comparison of bottom-up proteomic sample preparation for early-stage Xenopus laevis embryos. Anal. Bioanal. Chem. 408, 4743–4749. https://doi.org/10.1007/s00216-016-9564-2.
Pike, S., Matthes, M.S., McSteen, P., Gassmann, W., 2019. Using Xenopus laevis oocytes to functionally characterize plant transporters. Curr. Protoc. Plant Biol. 4, e20087 https://doi.org/10.1002/cppb.20087.
Pizzi, F., Gnoni, A., Ciani, E., Dell'Aquila, M.E., 2017. Exposure to cadmium during in vitro maturation at environmental nanomolar levels impairs oocyte fertilization through oxidative damage: a large animal model study. Reprod. Toxicol. 69, 132–145. https://doi.org/10.1016/j.reprotox.2017.02.005.
Prabhat, K.R., Sang, S.L., Ming, Z., Yiu, F.T., Ki-Hyun, K., 2019. Heavy metals in food crops: Health risks, fate, mechanisms, and management. Environment International 125, 365–385. https://doi.org/10.1016/j.envint.2019.01.067.
Radi, R., Turrens, J.F., Chang, L.Y., Bush, K.M., Crapo, J.D., Freeman, B.A., 1991. Detection of catalase in rat heart mitochondria. J. Biol. Chem. 266, 22028–22034.
Rangkadilok, N., Siripriwon, P., Nookabkaew, S., Suriyo, T., Satayavivad, J., 2015. Arsenic, cadmium, and manganese levels in shellfish from Map Ta Phut, an industrial area in Thailand, and the potential toxic effects on human cells. Arch. Environ. Contam. Toxicol. 68, 169–180. https://doi.org/10.1007/s00244-014-0054-2.
Rizzo, M.A., Adorni, L., Montorfano, G., Rossi, F., Berra, B., 2007. Antioxidant metabolism of Xenopus laevis embryos during the first days of development. Comp. Biochem. Physiol. B: Biochem. Mol. Biol. 146 (1), 94–100. https://doi.org/10.1016/j.cbpb.2006.09.009.
Robert, J., Ohta, Y., 2009. Comparative and developmental study of the immune system in Xenopus. Dev. Dyn. 238, 1249–1270. https://doi.org/10.1002/dvdy.21891.
Rodríguez-Sánchez, R., Ortiz-Butrón, R., Blas-Valdivia, V., Hernández-García, A., Cano-Europa, E., 2012. Phycobiliproteins or C-phycocyanin of Arthrospira (Spirulina) maxima protect against HgCl2-caused oxidative stress and renal damage. Food Chem. 135, 2359–2365. https://doi.org/10.1016/j.foodchem.2012.07.063.
Salnikova, E.V., Burtseva, T.I., Skalnaya, M.G., Skalny, A.V., Tinkov, A.A., 2018. Copper and zinc levels in soil, water, wheat, and hair of inhabitants of three areas of the Orenburg region, Russia. Environ. Res. 166, 158–166. https://doi.org/10.1016/J.ENVRES.2018.05.028.
Sant, K.E., Hansen, J.M., Williams, L.M., Tran, N.L., Goldstone, J.V., Stegeman, J.J., Hahn, M.E., Timme-Laragy, A., 2017. The role of Nrf1 and Nrf2 in the regulation of glutathione and redox dynamics in the developing zebrafish embryo. Redox Biol. 13, 207–218. https://doi.org/10.1016/j.redox.2017.05.023.
Sayed, A.E.H., Authman, M.M.N., 2018. The protective role of Spirulina platensis to alleviate the sodium dodecyl sulfate toxic effects in the catfish Clarias gariepinus (Burchell, 1822). Ecotoxicol. Environ. Saf. 163, 136–144. https://doi.org/10.1016/j.ecoenv.2018.07.060.
Sharma, B., Patiño, R., 2009. Effects of cadmium on growth, metamorphosis and gonadal sex differentiation in tadpoles of the African clawed frog, Xenopus laevis. Chemosphere 76, 1048–1055. https://doi.org/10.1016/j.chemosphere.2009.04.043.
Shirriff, C.S., Heikkila, J.J., 2017. Characterization of cadmium chloride-induced BiP accumulation in Xenopus laevis A6 kidney epithelial cells. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 191, 117–128. https://doi.org/10.1016/j.cbpc.2016.10.003.
Slaby, S., Lemière, S., Hanotel, J., Lescuyer, A., Demuynck, S., Bodart, J.-F., Leprêtre, A., Marin, M., 2016. Cadmium but not lead exposure affects Xenopus laevis fertilization and embryo cleavage. Aquat. Toxicol. 177, 1–7. https://doi.org/10.1016/j.aquatox.2016.05.003.
Slaby, S., Hanotel, J., Marchand, G., Lescuyer, A., Bodart, J.F., Leprêtre, A., Lemière, S., Marin, M., 2017. Maturation of Xenopus laevis oocytes under cadmium and lead exposures: cell biology investigations. Aquat. Toxicol. 193, 105–110. https://doi.org/10.1016/j.aquatox.2017.10.009.
Stahl, W., Sies, H., 2003. Antioxidant activity of carotenoids. Mol. Asp. Med. 24, 345–351. https://doi.org/10.1016/S0098-2997(03)00030-X.
Sunderman, F.W.J., Plowman, M., Hopfer, S., 1991. Embryotoxicity and teratogenicity of cadmium chloride in Xenopus laevis, assayed by the FETAX procedure. Ann. Clin. Lab. Sci. 21, 381–391 (doi:0091-7370/91/1100-0381).
Szuroczki, D., Koprivnikar, J., Baker, R.L., 2016. Dietary antioxidants enhance immunocompetence in larval amphibians. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 201, 182–188. https://doi.org/10.1016/j.cbpa.2016.07.014.
Thompson, J., Bannigan, J., 2008. Cadmium: toxic effects on the reproductive system and the embryo. Reprod. Toxicol. 25, 304–315. https://doi.org/10.1016/j.reprotox.2008.02.001.
Timme-Laragy, A.R., Hahn, M.E., Hansen, J.M., Rastogi, A., Roy, M.A., 2018. Redox stress and signaling during vertebrate embryonic development: regulation and

responses. Semin. Cell Dev. Biol. 80, 17–28. https://doi.org/10.1016/j.semcdb.2017.09.019.

Valavanidis, A., Vlahogianni, T., Dassenakis, M., Scoullos, M., 2006. Molecular biomarkers of oxidative stress in aquatic organisms in relation to toxic environmental pollutants. Ecotoxicol. Environ. Saf. 64, 178–189. https://doi.org/10.1016/j.ecoenv.2005.03.013.

Wang, Y., Fang, J., Leonard, S.S., Rao, K.M.K., 2004. Cadmium inhibits the electron transfer chain and induces reactive oxygen species. Free Radic. Biol. Med. 36, 1434–1443. https://doi.org/10.1016/j.freeradbiomed.2004.03.010.

Wu, Q., Liu, L., Miron, A., Klímová, B., Wan, D., 2016. The antioxidant, immunomodulatory, and anti-inflammatory activities of Spirulina: an overview. Arch. Toxicol. https://doi.org/10.1007/s00204-016-1744-5.

Wu, C., Zhang, Y., Chai, L., Wang, H., 2017a. Oxidative stress, endocrine disruption, and malformation of Bufo gargarizans embryo exposed to sub-lethal cadmium concentrations. Environ. Toxicol. Pharmacol. 49, 97–104. https://doi.org/10.1016/j.etap.2016.12.005.

Wu, C., Zhang, Y., Chai, L., Wang, H., 2017b. Histological changes, lipid metabolism and oxidative stress in the liver of Bufo gargarizans exposed to cadmium concentrations. Chemosphere 179, 337–346. https://doi.org/10.1016/j.chemosphere.2017.03.131.

Xia, L., Chen, S., Dahms, H.U., Ying, X., Peng, X., 2016. Cadmium induced oxidative damage and apoptosis in the hepatopancreas of Meretrix meretrix. Ecotoxicology 25, 959–969. https://doi.org/10.1007/s10646-016-1653-7.

Yan, S.G., Zhu, L.P., Su, H.N., Zhang, X.Y., Chen, X.L., Zhou, B.C., Zhang, Y.Z., 2011. Single-step chromatography for simultaneous purification of C-phycocyanin and allophycocyanin with high purity and recovery from Spirulina (Arthrospira) platensis. J. Appl. Phycol. 23, 1–6. https://doi.org/10.1007/s10811-010-9525-7.

Zhang, H., Cao, H., Meng, Y., Jin, G., Zhu, M., 2012. The toxicity of cadmium (Cd2+) towards embryos and pro-larva of soldatov's catfish (Silurus soldatovi). Ecotoxicol. Environ. Saf. 80, 258–265. https://doi.org/10.1016/j.ecoenv.2012.03.013.