Assessment of Adsorption and Removal Efficacy of Spirulina Powder for Strontium and Thallium

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Abstract

A nuclear accident or incident releases a large number of radionuclides. The most important fission produced radionuclides are cesium, strontium, iodine, and thallium. It enters the body through ingestion, inhalation and skin, and accumulates in tissues and organs. Thus, it is extremely important to remove these radionuclides as soon as possible. In concern of this, the present study was envisaged to evaluate the adsorption and removal efficacy of *Spirulina* for radionuclides viz., cobalt (Co), strontium (Sr), barium (Ba), cesium (Cs) and thallium (Tl). The adsorption efficacy was investigated in terms of weight of *Spirulina* (0.1 g), contact time (3h), metal ion concentration (10-100 mg L^{-1}), and simulated physiological fluids viz., simulated gastric (SGF) and intestinal fluid (SIF). The removal efficacy of *Spirulina* was evaluated for strontium and thallium in mice. The strontium and thallium content in major tissues, urine and faeces were estimated. The maximum binding capacity of *Spirulina* for Co^{+2}, Sr^{+2}, Ba^{+2}, Cs^{+1} and Tl^{+1} was found to be 3.774, 0.655, 0.337, 1.328 and 0.129 mg g^{-1} in water, respectively, and 0.838, 0.575, 0.697, 0.334 and 6.098 mg g^{-1} in SGF, respectively, whereas in SIF it was found to be 18.182, 0.329 and 1.203 mg g^{-1} for Sr^{+2}, Cs^{+1} and Tl^{+1}, respectively. *Spirulina* significantly reduced the whole body retention of strontium and thallium and enhanced their excretion through urine and faeces. In conclusion, pulverized *Spirulina* showed potential adsorption efficiency and may be used as a cost-effective, efficient and non-toxic agent for removal of strontium and thallium from body.

1. Introduction

Radioactive contamination of the environment has occurred not only through use of radiological dispersal devices (dirty bomb) but also through other means like destruction of nuclear reactors or by virtue of an industrial or military nuclear accident. The radioactive contaminants released during any accident or incident includes cesium-137, strontium-90, iodine-131, cobalt-60, americium-241 etc. (FDA, 2006). Barium-141, Cesium-137, and strontium-90 are produced from the nuclear fission of uranium and plutonium. Cesium-137 is decays into barium-137. With such an extensive use of radionuclides there is an increased possibility of humans getting exposed to them leading to external and/or internal contamination. A nuclear disaster leads to both external and internal contamination of the radioisotopes. External contamination is associated with the short-term effects of radiations. It includes the contamination of skin surface, foodstuff, water and gamma radiations from the spread of radioisotopes in the atmosphere. About 95% of decontamination can be achieved by removal of clothing, and washing with soap or detergent and water (FDA, 2006).

Internal contamination occurs through ingestion, inhalation or absorption through skin contact of radioactive materials. Exposure to radioactive or nonradioactive materials via ingestion is a major exposure pathway to human and animals. Many factors, such as chemical nature, and physical and biological half life of radionuclides or metal ions affect its absorption, distribution, metabolism and elimination in human body. Once inside the body, radionuclides pose major health problems due to their emission characteristics in the form of alpha, beta particles or gamma rays. Although they are present in micro-quantity, which is far below the metal toxicity level but the radio-toxicity (toxicity due to emitted radiation) is very high. Although some metal ions, such as cobalt and iron are essential for maintaining normal physiological functions, but at higher concentration or their radioactive isotopes leads to poisoning. The metal ions like mercury, cesium, thallium and strontium have the greatest potential to cause harm on account of their extensive use. Following entry into the blood, most of the ions are excreted through kidney and some of them get accumulated into their target organs or tissues. Once inside the body they affect liver, kidney, hematopoietic and nervous system. Further, it causes various disorders like cardiac irregularities, anxiety, tremor and paralysis. Its presence in bones can cause bone cancer, cancer of nearby tissues, and leukemia. After entering the body, biological behavior of cesium and thallium is similar to that of potassium and is excreted by the bile in enterohepatic recirculation (Avery 1995) whereas strontium behaves like calcium (EPA 2017). After entering the body, most of the cesium-137 (biological half life, 70–110 days) and thallium (biological half life, 8–10 days) gets deposited into soft tissues. After Sr-90 is absorbed, it acts like Ca^{+2} and is readily absorbed into bones and teeth, where it can cause cancer of bone, bone marrow and soft tissue surrounding bone (ATSDR, 2014). The removal of toxic metals and radionuclides from body is required to prevent its accumulation in organs or tissues.

Potential agents avert the adverse effects of toxic metal ions by removing them from the body. Chelating and diluting agent helps to accelerate the removal of toxic metal ions through the kidneys (Queiroz et al., 2003; Klaassen, 1996; Gurer and Ercal, 2000). Adsorption over conventional methods such as chelation, reverse osmosis and hemoperfusion is an easy and advantageous process for the removal of radionuclides and toxic metal ions. The finding of new technologies has drawn attention to algae based adsorbents or agents that can remove radionuclides/toxic metals from the body. However, systematic studies are needed to establish a safe and efficacious alga based adsorbent/agents over conventional agents. Algae like *Spirulina* and *Chlorella* long been associated with detoxification, specifically the detoxification of toxic/heavy metals. They are highly effective in binding and elimination of toxic metals like lead, cadmium, chromium, mercury, strontium, thallium etc. (Sandau et al., 1996; Rangsayatorn et al., 2004; Chojnacka et al., 2004, 2005; Yadav et al. 2020, 2021a, 2021b).

Spirulina is blue-green algae, consumed as dietary food supplement in pulverized form. In addition to its high nutritional value, it is reported as an excellent detoxifying agent (Chojnacka et al., 2004). Therefore, the present study was undertaken to evaluate the potential of *Spirulina* for adsorption and removal of non-radioactive isotopes of radionuclides. *Spirulina* is a genus of blue-green algae belonging to the family of *Oscillatoriaceae*. *Spirulina* is a filamentous *cyanobacterium* composed of individual cells (about 8 µm diameter), which grows in subtropical, alkaline lakes with a temperature optimum of about 35°C (Masojídek and Torzillo, 2014). *Spirulina* is the commercial name refers to the dried biomass of *Spirulina platensis* (also known as *Arthrospira platensis*) (Gershwin and Belay, 2007). The two species which are most commonly utilized are *Spirulina platensis* and *Spirulina maxima*. Dried *Spirulina* contains about 5% water, 55–60% protein, 10–20% carbohydrates, 9–14% lipids, 0.8–1.5% chlorophyll, minerals and vitamins (De Smet, 1997; Khan et al., 2005).

In addition to its ability to bind to toxic metals or radionuclides, *Spirulina* also contains a lot of important vitamins and minerals that could help during detoxification. *Spirulina* contains a variety of metal-binding functional groups such as carboxyl, amino, phosphoryl, hydroxyl and carbonyl groups, which has high affinity towards various metal ions (Chojnacka et al., 2005; Fang et al., 2011). Therefore, pulverized (broken cell wall) *Spirulina platensis* were used in this study to bind/adsorb and remove various metal ions viz., cobalt, strontium, barium, cesium and thallium. Binding efficiency was determined in terms of adsorbent weight, contact time, pH and metal ions concentration. The maximum binding capacity of *Spirulina* for cobalt, strontium, barium, cesium and thallium were determined by using Langmuir and Freundlich adsorption isotherm models (Ayawei et al., 2017; Yadav et al., 2020). In addition to this, the *in vivo* removal efficacy of *Spirulina* was evaluated for strontium and thallium in Swiss albino mice.

2. Materials And Methods

2.1 Drugs and Chemicals

Spirulina powder (source; *Spirulina platensis*), Batch No. GNH SLP 4/18, was obtained from Genius Nature Herbs Pvt. Ltd., Coimbatore, India. Chloride salts of cobalt (Lot No. QL32B-GN), strontium (Batch No. 1348524), barium (Batch No. 110310), and cesium (Lot No. MKCJ4068) and thallium (Lot No. MKCL1081) were procured from TCI Chemicals (India) Pvt. Ltd., Sisco Research Laboratories Pvt. Ltd. India, Central Drug House (P) Ltd. India, and Sigma-Aldrich USA, respectively. The atomic absorption spectroscopy (AAS) standard solution of strontium, barium, cesium and thallium was procured from High purity laboratory chemicals Pvt. Ltd., Mumbai, India and AAS standard solution of cobalt was procured from Alfa Aesar, USA. These AAS standard solutions contain metal ion concentration of 1000 ± 10 mg L^{-1}. All other chemicals used in the experiment were of the highest grade commercially available.

2.2 FTIR Spectrometric Analysis of *Spirulina* powder

The dried *Spiruliina* powder was processed to record IR spectrum using Attenuated total reflection -Fourier transform infrared spectrometry (ATR-FTIR spectrometry, Perkin Elmer Model no-1400). The spectra range 500 to 4000 cm^{-1} was used for scanning.

2.3 Assessment of *in vitro* binding/adsorption efficacy of S*pirulina* for cobalt, strontium, barium, cesium and thallium

The batch experiment was conducted to determine the binding or adsorption capacity of *Spirulina* for cobalt, strontium, barium, cesium and thallium in each parameter. Weighed quantity of *Spirulina* powder (0.1 g) was mixed thoroughly in water and simulated physiological solutions. Stock solutions of cobalt (100 mg L^{-1}), strontium (100 mg L^{-1}), barium (100 mg L^{-1}), cesium (100 mg L^{-1}) and thallium (100 mg L^{-1}) were prepared by dissolving 0.4037 g cobalt chloride, 0.30429 g strontium chloride, 0.124 g barium chloride, 0.1267 g cesium chloride and 0.173 g thallium chloride in 1000 mL Millipore water. These stock solutions were used to assess the adsorption capacity of *Spirulina* for metal ions. The *in vitro* adsorption efficiency of *Spirulina* for cobalt, strontium, barium, cesium and thallium was studied in terms of weight of adsorbent (10 g L^{-1}), contact time (3h), simulated gastric physiological conditions (SGF and SIF) and metal ion concentration (10–100 mg L^{-1}). The batch experiment was conducted to determine the binding or adsorption capacity of *Spirulina* for cobalt, strontium, barium, cesium and thallium in each parameter. The percentage adsorption of metals on *Spirulina* were determined by using formula (Supplementary material, Figure S1-S5)

$$\%Adsorption = \frac{C_i - C_f}{C_i} x100$$

The adsorption capacity of *Spirulina* (mg g^{-1}) for these metal ions was determined using the formula (Yadav et al. 2020):

$$Adsorptioncapacity\left(mgg^{-1}\right) = \left(C_i - C_f\right) x \frac{v}{w}$$

Where, C_i = initial concentration of metal at time 0, C_f = final concentration of metal at time t, v = volume of medium in L and w = weight of the adsorbent (*Spirulina* powder) in g.

The simulated physiological fluids viz. simulated gastric fluid (SGF, pH 1.5) without pepsin and simulated intestinal fluid (SIF, pH 6.8) without pancreatin were prepared as per United States Pharmacopeia (USP-NF 2002, 2003; Stippler et al. 2004). Weighed amount of *Spirulina* (0.1 g) was introduced in 15 mL centrifuge tube containing 10 mL solution of cobalt, strontium, barium, cesium or thallium in SGF, SIF and water. The adsorption efficiency of *Spirulina* was also investigated against different concentration of these metal ions. The *Spirulina* (10 g L^{-1}) was incubated for 3h with these metal ions (10–100 mg L^{-1}) in SGF, SIF and water. The samples were collected at equilibrium time and analyzed for metal content. The filtered samples were appropriately diluted with 5% HNO_3 solution and analyzed for cobalt, strontium, barium, cesium and thallium contents using AAS (FAAS, Labindia AA 8000, Mumbai, India). The adsorption capacity of *Spirulina* was determined for each concentration of cobalt, strontium, barium, cesium or thallium in SGF, SIF and water. The adsorption capacity of *Spirulina* for cobalt, strontium, barium, cesium and thallium in SGF and SIF were compared with water.

2.3.1 Adsorption isotherm models

The maximum binding capacity (MBC) of *Spirulina* was assessed against different concentration of cobalt, strontium, barium, cesium and thallium (10–100 mg L^{-1}) in water, SGF and SIF. The MBC of *Spirulina* for these metal ions (mg g^{-1}) were determined by subjecting the data to Langmuir and Freundlich adsorption isotherm models. Langmuir and Freundlich adsorption isotherm models were used to quantify the MBC and energy of adsorption of *Spirulina* for different metal ions. In Langmuir and Freundlich adsorption isotherm models, regression coefficient (R^2) is a goodness-of-fit measure for linear regression models. The Langmuir adsorption isotherm model assumes containing a finite number of adsorption sites. The linear equation of Langmuir adsorption isotherm is represented by:

$$\frac{C_e}{q_e} = \frac{1}{q_m K_L} + \frac{C_e}{q_m}$$

Where C_e = concentration of free metal ion at equilibrium (mg L^{-1}), q_e = amount of metal ion adsorbed at equilibrium (mg g^{-1}), q_m = maximum binding capacity of adsorbent (mg g^{-1}) and K_L = constant related to the energy of adsorption (L mg^{-1}). Plot the line between C_e/q_e *vs* C_e and from which the constants q_m and K_L calculated. The X axis represents the concentration of free metal (mg L^{-1}) in the solution at equilibrium. The Y axis represents the ratio of the concentration of free metal (mg L^{-1}) versus the bound metal (mg g^{-1}) at equilibrium (Faustino et al. 2008; Ayawei et al. 2017; Yadav et al. 2020)

K_L can be used to determine the linearity of the adsorption (R_L) using formula

$$R_L = \frac{1}{1 + C_i K_L}$$

Where, C_i is the initial concentration of the adsorbate (10 mg L^{-1}). When the R_L value is less than unity, adsorption is said to be favorable and when greater than unity it is considered unfavorable. Also, when R_L is equal to 0, the adsorption is irreversible, and the unity value represents the linearity of the adsorption.

The Freundlich adsorption isotherm model suggests the relative distribution of the energy and the heterogeneity of the adsorbate sites (Ayawei et al. 2017). The linearised Freundlich adsorption isotherm is represented by:

$$\ln q_e = \ln K_f + \frac{1}{n} \ln C_e$$

Where K_f (L mg^{-1}) is a Freundlich constant characteristic of a particular adsorption isotherm and n (dimensionless) is a Freundlich constant representing the adsorption intensity. A plot of $\ln q_e$ *vs* $\ln C_e$ would give a straight line from which constants K_f and n calculated (Yadav et al. 2020).

The plot of the Langmuir and Freundlich adsorption isotherm models gives the square of the correlation coefficient (R^2). The value of R^2 near unity represents the monolayer adsorption of the adsorbate on the adsorbent with the higher energy of adsorption.

2.4 Assessment of *in vivo* Removal Efficacy of *Spirulina* for Strontium and Thallium in Mice

2.4.1 Experimental animals and ethical approval

Swiss albino mice (25-35g) of either sex were selected for the assessment of *in-vivo* removal efficiency of *Spirulina*for cobalt, strontium, barium, cesium and thallium. The animals were acclimatized to standard laboratory conditions of temperature 22 ± 3°C with relative humidity 55 ± 5% under 12h light: 12h dark cycle. They were provided with regular standard feed and drinking water *ad libitum*. All the protocols and experiments were approved by the Institutional Animal Ethics Committee (IAEC approval no. ; INM/IAEC/2018/07) and conducted according to ethical guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi.

2.4.2 Experimental design

In vivo removal efficacy of *Spirulina* for strontium and thallium was assessed in Swiss albino mice. Mice were divided into six groups (n = 6). Three groups (n = 6) were selected for each metal ions. The experiment was conducted with strontium (30 mg kg^{-1}) and thallium (10 mg kg^{-1}) to determine the *in vivo* removal efficacy of *Spirulina* for these metal ions. In a preliminary study, we found that orally administered strontium dose less than 30 mg kg^{-1} were not detected in most of the soft tissues after 24 hours because most of the strontium was either excreted or accumulated in bone. Similar observations were found with a 30 mg kg^{-1}dose of strontium administered for a period of more than 24 hours. Therefore a 30 mg kg^{-1} strontium dose was selected for mice for a maximum duration of 24 h.

The group I-III received strontium chloride at dose of 30 mg kg^{-1} and treatment of *Spirulina* was given as follow

Group I served as experimental control for groups II-III, received strontium chloride (30 mg kg^{-1}) only,

Group II co-treated with *Spirulina* 500 mg/70 kg human equivalent dose (HED), and

Group III co-treated with *Spirulina* 1000 mg/70 kg HED.

The group IV-VI received thallium chloride at dose of 10 mg kg^{-1} and treatment of *Spirulina* was given as follow

Group IV served as experimental control for groups V-VI, received thallium chloride (10 mg kg^{-1}) only,

Group V co-treated with *Spirulina* 500 mg/70 kg HED, and

Group VI co-treated with *Spirulina* 1000 mg/70 kg HED.

The doses for mice were calculated by converting *Spirulina* human dose (500 and 1000 mg *Spirulina* /70 kg human) to animal dose (mg kg^{-1}) as described by Nair and Jacob (2016). All the doses were prepared in Millipore ultrapure water and administered once, orally and observed for 24h.

Urine and faecal collection

Immediately after dosing all animals were kept individually in metabolic cages with free access to drinking water and feed. Urine and faecal samples of 24 h were collected and measured for their volume and weight.

Blood collection

At the end of experiment, mice were anaesthetized and blood samples were collected from the retro-orbital plexus.

Organs/tissues collection

To determine the metal ion content in organs, the animals were sacrificed after blood collection. The major organs viz., kidney, liver, spleen, heart, brain, stomach, intestine, colon, muscle and bone from each animal were collected and weighed. The collected organs or tissues were carefully cleaned to remove excess tissues. Weighing approximately 0.1 g and placed in a glass tube and closed properly. The whole tissue retention in each animal was calculated as the sum of strontium or thallium levels in all collected tissues.

Digestion and analysis of samples

Weighed amount of urine, fecal, blood and tissue samples were digested using concentrated nitric acid in a glass test tube. To allow complete digestion, all the test tube were closed and placed in fume hood, and left for 2–3 days. The digested samples of blood, urine, faecal and organs were diluted with water and centrifuged using centrifuge machine (REMI R-12C Plus, India). The supernatant was collected and after appropriate dilutions, the digested samples were collected and analyzed for strontium and thallium content in blood, urine, faecal and major organs using graphite furnace atomic absorption spectrophotometer (GF-AAS, Labindia AA 8000, Mumbai, India).

2.5 Statistical Analysis

The results were expressed as mean ± SEM, (*in vitro* study, n = 3–4, and *in vivo* study, n = 6). The data were analyzed statistically using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego California USA). Statistical analysis was performed by one-way ANOVA followed by Tukey's post hoc test and two-way ANOVA followed by Bonferroni multiple comparison tests, wherever necessary. A value of $p < 0.05$ was considered significant in all cases.

3. Results

3.1 FTIR Spectrometric Analysis of *Spirulina* powder

FTIR spectrum and absorption peaks of *Spirulina* represent the respective functional groups and compounds (Fig. 1). C–H stretching of the methylene groups at 3279.84 cm^{-1} showed the presence of proteins and lipids. The peak at 2940.25 cm^{-1} represents the N–H stretch vibration of the secondary amide of the protein. The peak at 1641.35 cm^{-1} corresponds to the asymmetric C = O stretch vibration and/or the aromatic C = C stretch vibration. The peak at 1537.69 cm^{-1} corresponds to N–O stretching of nitro compounds. The peak at 1394.04 cm^{-1}, assigned to the O–H bending of the carboxylic acid, indicates the presence of amino acids and fatty acids. The presence of alkyl or aryl compounds was indicated by C–O stretching at 1240.41 cm^{-1}. The carbohydrate absorption bands due to C–OH stretching of polysaccharides at 1028.61cm^{-1}.

3.2 Assessment of *in vitro* binding/adsorption efficacy of S*pirulina* for cobalt, strontium, barium, cesium and thallium

3.2.1 Assessment of *in vitro* binding/adsorption efficacy of *Spirulina* for cobalt

The adsorption capacity of *Spirulina* increased with increasing the amount of cobalt (10–100 mg L^{-1}) in water (0.298 ± 0.01–1.979 ± 0.067 mg g^{-1}) and SGF (0.227 ± 0.014–0.706 ± 0.040 mg g^{-1}) (Fig. 2). The results shown that, the adsorption capacity of *Spirulina* was high ($p < 0.001$) in water compared to SGF. The addition of cobalt above 50 mg L^{-1} leads to a plateau which indicates the saturation of the binding sides. The graphs of Langmuir and Freundlich adsorption isotherm models are represented in Fig. 3. These adsorption isotherm models were applied for determination of MBC of *Spirulina* for cobalt in water and SGF. The Langmuir and Freundlich models (Fig. 3A and B) showed the best fit model, indicating *Spirulina* has finite identical site to form a monolayer adsorption with cobalt with higher intensity and energy of adsorption. Table 1 represents the parameters of Langmuir and Freundlich adsorption isotherm models. The MBC of *Spirulina* for cobalt was found to be 3.774 and 0.838 mg g^{-1} in water and SGF, respectively. Since cobalt precipitates in SIF, the adsorption capacity of *Spirulina* for cobalt was not evaluated.

3.2.2 Assessment of i*n vitro* binding/adsorption efficacy of *Spirulina* for strontium

Figure 4 illustrated the efficiency of *Spirulina* (10 g L^{-1}) against different concentration of strontium (10–100 mg L^{-1}) in water and simulated physiological fluids (SGF and SIF). The adsorption capacity of *Spirulina* increased with increasing amount of strontium in water (0.296 ± 0.041–0.989 ± 0.006 mg g^{-1}), SGF (0.192 ± 0.025–0.437 ± 0.048 mg g^{-1}) and SIF (0.284 ± 0.027–2.342 ± 0.023 mg g^{-1}). At higher concentration of strontium (20–100 mg L^{-1}), the adsorption capacity of *Spirulina* significantly increased ($p < 0.01$-$p < 0.001$) in order of SIF > water > SGF, whereas at low strontium concentration (10 mg L^{-1}), adsorption capacity was same in all media (Fig. 4). At equilibrium time, the adsorption capacity of *Spirulina* increased with increased concentration of strontium in water and SIF. However, in SGF the adsorption capacity was minimal compared to SIF and water. In SGF, the addition of strontium after 50 mg L^{-1} showed a plateau phase of adsorption on *Spirulina*, indicating saturation of the binding sites. Figure 5 represents the graphs of Langmuir and Freundlich adsorption isotherm models for determination of MBC of *Spirulina* for strontium in water, SGF and SIF. The Freundlich model (Fig. 5B) showed the best fit than Langmuir model (Fig. 5A), indicating *Spirulina* did not have identical site to form a monolayer adsorption with strontium but intensity and energy of adsorption was high. The MBC of *Spirulina* for strontium was found to be 1.276, 0.575 and 18.182 mg g^{-1} in water, SGF and SIF, respectively (Table 1).

3.2.3 Assessment of i*n vitro* binding/adsorption efficacy of *Spirulina* for barium

Adsorption of barium on *Spirulina* in water and simulated gastric fluid (SGF) is illustrated in Fig. 6. At higher concentration (50–100 mg L^{-1}) of barium the adsorption capacity of *Spirulina* significantly increased ($p < 0.01$-$p < 0.001$) in SGF when compared to water. At low concentration (10–25 mg L^{-1}) of barium, insignificant differences observed between water and SGF. The adsorption capacity of *Spirulina* increased with increasing the amount of barium (10–100 mg L^{-1}) in water (0.124 ± 0.00–0.286 ± 0.06 mg g^{-1}) and SGF (0.202 ± 0.04–0.567 ± 0.04 mg g^{-1}) (Fig. 6). Optimal adsorption on *Spirulina* was achieved at 50 mg L^{-1} barium in water and SGF, and further addition of barium showed no significant difference in adsorption. Figure 7A and B represents the graphs of Langmuir and Freundlich adsorption isotherm models for determination of MBC of *Spirulina* for barium in water and SGF. Both the adsorption isotherm models viz., Langmuir (Fig. 7A) and Freundlich models (Fig. 7B) were best fitted in water and SGF, indicates monolayer adsorption of barium on *Spirulina* with high intensity and energy of adsorption. The MBC of *Spirulina* for barium was found to be 0.337 and 0.697 mg g^{-1} in water and SGF, respectively (Table 1). Since barium precipitates in SIF, the adsorption capacity of *Spirulina* for barium was not evaluated.

3.2.4 Assessment of i*n vitro* binding/adsorption efficacy of *Spirulina* for cesium

The adsorption capacity of *Spirulina* for cesium was increased with increasing the concentration of cesium. A significant increased adsorption capacity of *Spirulina* for cesium was observed in water compared to SGF and SIF. At higher concentration of cesium the adsorption capacity of *Spirulina* significantly increased ($p < 0.01$-$p < 0.001$) in water when compared to SGF and SIF. At low concentration of cesium, insignificant differences observed between water, SGF and SIF. No significant differences were observed in the adsorption capacity of *Spirulina* to cesium in SGF and SIF at all concentration levels. The adsorption capacity of *Spirulina* for cesium (10–100 mg L^{-1}) in water, SGF and SIF was 0.062 ± 0.00–0.452 ± 0.02, 0.104 ± 0.008–0.279 ± 0.026 and 0.056 ± 0.016–0.230 ± 0.031 mg g^{-1}, respectively (Fig. 8). Figure 9 represents the graphs of Langmuir and Freundlich adsorption isotherm models for determination of MBC of *Spirulina* for cesium in water, SGF and SIF. The Langmuir model (Fig. 9A) showed the best fit in water and SGF than SIF, whereas Freundlich model (Fig. 9B) was best fitted in water, SGF and SIF. The result indicates, change in pH affects the sites present for the adsorption of cesium on *Spirulina* to form a monolayer adsorption. The MBC of *Spirulina* for cesium was found to be 1.328, 0.334 and 0.329 mg g^{-1} in water, SGF and SIF, respectively (Table 1).

3.2.5 Assessment of i*n vitro* binding/adsorption efficacy of *Spirulina* for thallium

The adsorption capacity of *Spirulina* for thallium increased with increasing the concentration of thallium. There was significant increase in adsorption capacity of *Spirulina* for thallium in SGF and SIF when compared to water. At higher concentration of thallium the adsorption capacity of *Spirulina* significantly increased ($p < 0.001$) in SGF and SIF when compared to water. At lower concentration of thallium, insignificant differences observed between water and SIF. The adsorption capacity of *Spirulina* for thallium (10–100 mg L^{-1}) in water, SGF and SIF was found to be 0.199 ± 0.020–0.477 ± 0.001, 0.332 ± 0.036–2.109 ± 0.025 and 0.051 ± 0.005–0.690 ± 0.014 mg g^{-1}, respectively (Fig. 10). Figure 11A and B represents the graphs of Langmuir and Freundlich adsorption isotherm models for determination of MBC of *Spirulina* for thallium in water, SGF and SIF. The Langmuir model (Fig. 11A) showed the best fit in water than SGF and SIF. Whereas the Freundlich model (Fig. 11B) showed the best fit in SGF and SIF than water. The results indicate, pH of medium affects the adsorption of thallium on *Spirulina*. This revealed the homogeneous sorption of

thallium in water and heterogeneous sorption in SGF and SIF. The MBC of *Spirulina* for thallium was found to be 0.129, 6.098 and 1.203 mg g^{-1} in water, SGF and SIF, respectively (Table 1).

Table 1
Langmuir and Freundlich adsorption isotherm parameters for adsorption of cobalt, strontium, barium, cesium and thallium on *Spirulina* in different physiological solutions

Isotherm model	Parameters	Cobalt		Strontium			Barium		Cesium			Thallium		
		Water	**SGF**	**Water**	**SGF**	**SIF**	**Water**	**SGF**	**Water**	**SGF**	**SIF**	**Water**	**SGF**	**SIF**
Langmuir	q_m (mg g^{-1})	**3.774**	**0.838**	**1.276**	**0.575**	**18.182**	**0.337**	**0.697**	**1.328**	**0.334**	**0.329**	**0.129**	**6.098**	**1.203**
	K_L (L mg^{-1})	0.137	0.055	0.031	0.036	0.002	0.050	0.037	0.005	0.042	0.016	0.306	0.006	0.014
	R_L	0.422	0.645	0.763	0.735	0.980	0.667	0.730	0.952	0.704	0.862	0.246	0.943	0.877
	R^2	0.967	0.980	0.958	0.934	0.468	0.987	0.979	0.955	0.979	0.803	0.984	0.934	0.923
Freundlich	n	1.332	2.227	2.105	2.320	1.070	2.849	2.299	1.186	2.545	1.751	21.27	1.186	1.515
	K_f (L mg^{-1})	0.079	0.102	0.113	0.065	0.040	0.057	0.078	0.010	0.015	0.015	0.119	0.053	0.035
	R^2	0.987	0.882	0.995	0.883	0.997	0.998	0.993	0.998	0.999	0.966	0.894	0.998	0.995

Where, q_m- maximum binding capacity (MBC), K_L- Langmuir constant (energy of adsorption), *R_L- Linearity of the adsorption, **n**- Freundlich constants (adsorption intensity), K_f- affinity of the binding sites, #R^2- square of the correlation coefficient. **SGF**- simulated gastric fluid, **SIF**- simulated intestinal fluid.

*When the R_L value is less than unity, adsorption is said to be favorable and when greater than unity it is considered unfavorable. Also, when R_L is equal to 0, the adsorption is irreversible, and the unity value represents the linearity of the adsorption. #In Langmuir and Freundlich adsorption isotherm models, value of R^2 near unity represents the monolayer adsorption of the adsorbate on the adsorbent with the higher energy of adsorption.

3.3 Assessment of *in vivo* Removal Efficacy of *Spirulina* for Strontium and Thallium in Mice

3.3.1 Assessment of *in vivo* removal efficacy of *Spirulina* for strontium in mice

The oral administration of strontium chloride (30 mg kg^{-1}) to control mice (group I) showed significant elevation of strontium level in kidney, liver, stomach, intestine, colon, spleen, heart, brain and bone compared to *Spirulina* treated mice (group II and III). No significant differences were observed in thallium levels in the muscle and blood of the *Spirulina* treated and control groups. The levels of strontium per gram of tissue are depicted in Table 2. The treatment of *Spirulina* 500 and 1000 mg/70 kg HED showed significant decrease ($p < 0.01$ - $p < 0.001$) in the level of strontium in kidney, liver, stomach, intestine, colon, spleen, heart, brain and bone compared to control. The *Spirulina* 500 and 1000 mg/70 kg HED treated group did not show any significant decrease in level of strontium in muscle and blood compared to control. The whole tissue retention in each animal was calculated as the sum of strontium content in all tissues. The removal efficacy of *Spirulina* was evaluated by determining the total excretion of strontium in urine and faeces. The whole tissue retention was significantly low ($p < 0.001$) in animals treated with *Spirulina* 500 and 1000 mg/70 kg HED compared to control group (Fig. 12). The level of strontium in urine and faeces significantly increased ($p < 0.05$-$p < 0.001$) in animals treated with *Spirulina* 500 and 1000 mg/70 kg HED compared to control (Fig. 13A and B).

In present study, most of the orally administered strontium was accumulated in the bone (> 90%) in mice of control group (Fig. 14). After absorption, strontium get also accumulated in other soft tissues in a very small amount as 2.7% in kidney, 0.4% in liver, 0.4% in stomach, 0.4% in intestine, 1.3% in colon, 1.5% in spleen, 2.1% in heart, 0.4% in brain, 0.2% in muscle and 0.1% in blood. Treatment with *Spirulina* 500 and 1000 mg/70 kg HED resulted in a reduction in whole tissue retention of strontium by 48.8 and 74.1%, respectively, of which 45.5 and 72.6% decreased in bone only as compared to the control group. In addition, *Spirulina* 500 and 1000 mg/70 kg HED significantly increased the elimination of strontium in urine by 38.10 and 80.34%, respectively, and in faecal by 77.53 and 162.95%, respectively, as compared to the control group.

3.3.2 Assessment of *in vivo* removal efficacy of *Spirulina* for thallium in mice

Thallium chloride (10 mg kg^{-1}) administered orally to group IV-VI. Administration of thallium chloride (10 mg kg^{-1}) to control animals (Group IV) showed significant increased level of thallium kidney, liver, stomach, colon, heart, brain, muscles and bone compared to *Spirulina* treated groups. The

levels of thallium per gram of tissue are depicted in Table 2. *Spirulina* treatment at dose of 1000 mg/70 kg HED (Group VI) significantly reduced ($p < 0.05$ - $p < 0.001$) the level of thallium in kidney, Liver, stomach, colon, heart, brain, muscles and bone compared to control. Treatment with *Spirulina* 500 mg/70 kg HED Group V) was only effective ($p < 0.01$) in reducing thallium levels in the brain and muscles compared to controls. *Spirulina* at both doses showed insignificant decrease in thallium levels in intestine, spleen, muscles and blood compared to the control group (Table 2). The whole tissue retention significantly decreased ($p < 0.01$ - $p < 0.001$) in *Spirulina* (500 and 1000 mg/70 kg HED) treated groups when compared to control group (Fig. 15). The treatment of *Spirulina* 500 mg/70 kg HED showed insignificant increase in thallium level in urine, whereas *Spirulina* 1000 mg/70 kg HED treatment showed significant increase ($p < 0.05$) in urine thallium level compared to control (Fig. 16A). Thallium excretion through faeces was significantly higher ($p < 0.001$) at 1000 mg/70 kg HED dose of *Spirulina* compared to control (Fig. 16B). *Spirulina* at dose of 500 mg/70 kg HED did not show significant increase in faecal thallium level. This reveals that the *Spirulina* 1000 mg/70 kg HED treated group had lower thallium retention in tissues and higher excretion in urine and faeces.

Whole tissue retention in control group was represented as the percentage deposition of thallium in different tissues such as kidney (27.0%), liver (6.4%), stomach (6.0%), intestine (8.5%), colon (9.1%), spleen (10.0%), heart (8.4%), brain (4.3%), muscle (9.2%), bone (10.3%) and blood (0.6%) (Fig. 17). After treatment with *Spirulina* 1000 mg/70 kg HED a significant reduction was observed in deposition of thallium in kidney, liver, stomach, colon, heart, brain, muscle and bone by 32.7, 21.3, 31.4, 21.4, 24.4, 39.2, 72.0 and 39.4%, respectively, whereas *Spirulina* 500 mg/70 kg HED reduced 26.5 and 72.3% of thallium in brain and muscle only. After treatment with *Spirulina*500 and 1000 mg/70 kg HED the whole tissue retention decreased by 10.92 and 31.44%, respectively and excretion was increased in urine by 9.02 and 32.98%, respectively, and in faecal by 8.37 and 69.74%, respectively.

Table 2
Effect of *Spirulina* on tissue deposition of strontium and thallium in mice

Tissues	Strontium Content (μg g^{-1})			Thallium Content (μg g^{-1})		
	Group I	Group II	Group III	Group IV	Group V	Group VI
	Control	500	1000	Control	500	1000
Kidney	3.524 ± 0.195	0.290 ± 0.068^{c}	0.070 ± 0.028^{c}	9.003 ± 0.212	8.854 ± 0.256	6.055 ± 0.234$^{\$}$
Liver	0.576 ± 0.059	0.131 ± 0.040^{c}	0.076 ± 0.035^{c}	2.138 ± 0.042	2.070 ± 0.108	1.683 ± 0.061$^{\#}$
Stomach	0.544 ± 0.053	0.269 ± 0.047^{b}	0.095 ± 0.023^{c}	2.002 ± 0.180	1.816 ± 0.079	1.37 ± 0.152$^{@}$
Intestine	0.467 ± 0.016	0.295 ± 0.034^{b}	0.089 ± 0.037^{c}	2.837 ± 0.104	2.845 ± 0.144	2.559 ± 0.243
Colon	1.749 ± 0.168	0.865 ± 0.140^{b}	0.547 ± 0.162^{c}	3.034 ± 0.082	2.855 ± 0.052	2.384 ± 0.078$^{\$}$
Spleen	1.928 ± 0.128	0.120 ± 0.042^{c}	0.071 ± 0.024^{c}	3.325 ± 0.307	3.134 ± 0.109	2.627 ± 0.152
Heart	2.721 ± 0.212	0.177 ± 0.072^{c}	0.145 ± 0.080^{c}	2.814 ± 0.105	2.785 ± 0.076	2.126 ± 0.082$^{\$}$
Brain	0.530 ± 0.023	0.123 ± 0.028^{c}	0.104 ± 0.036^{c}	1.447 ± 0.095	1.063 ± 0.036$^{\#}$	0.880 ± 0.070$^{\$}$
Muscle	0.231 ± 0.080	0.137 ± 0.066	0.120 ± 0.028	3.074 ± 0.495	0.853 ± 0.085$^{\$}$	0.860 ± 0.063$^{\$}$
Bone	117.8 ± 4.409	64.17 ± 8.325^{c}	31.29 ± 3.304^{c}	3.430 ± 0.137	3.179 ± 0.138	2.077 ± 0.140$^{\$}$
Blood	0.111 ± 0.010	0.049 ± 0.024	0.045 ± 0.029	0.209 ± 0.012	0.221 ± 0.053	0.215 ± 0.023

Values are expressed in mean ± SEM (n = 6). Significant values are $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$ compared to strontium control (Group IV, Sr 30 mg kg^{-1}) and $^{@}p < 0.05$, $^{\#}p < 0.01$, $^{\$}p < 0.001$ compared to thallium control (Group VII, Tl 10 mg kg^{-1}). *Spirulina* doses were given at 500 and 1000 mg/70 kg HED (denoted as 500 and 1000 in table).

4. Discussion

Spirulina showed a significant increase in the adsorption capacity to various metal ions with varying concentrations. An increase in the metal ion concentration showed a significant increase in the metal binding capacity of *Spirulina* in water, SGF and SIF. The MBC of *Spirulina* to cobalt, strontium, barium, thallium and cesium was found to be different in different physiological solutions. The MBC of *Spirulina* for different metal ions in water was ordered as Co > Cs > Sr > Ba > Tl, and in SGF it was ordered as Tl > Co > Ba > Sr > Cs. The MBC in SIF was ordered as Sr > Tl > Cs. Since cobalt and barium precipitates in SIF, the adsorption capacity of *Spirulina* for cobalt and barium was not evaluated in SIF. The linear regression curve plotted between cobalt, strontium, barium, cesium and thallium bound and unbound with *Spirulina* showed R^2 values close to one in water, SGF or SIF indicating monolayer adsorption. Furthermore the R_L value is less than unity which suggested that the adsorption of these metals on *Spirulina* is favorable (Table 1).

The results of *in vivo* studies suggested that almost all ingested strontium and thallium metal ions are immediately absorbed through the gastrointestinal tract. The absorption of ingested strontium through the gut was higher than that of thallium. Some of the absorbed metal ions are excreted through the kidneys and the rest are deposited in various tissues. In the present study, oral administration of strontium chloride (30 mg kg^{-1}) in mice showed rapid absorption and distributed throughout the body. Almost all strontium (> 90%) had accumulated in bone within 24 hours of administration and only a small amount (< 10%) was accumulated in other tissues or organs (Fig. 14). Strontium (Sr^{2+}) is a chemical analogue of Ca^{2+}, physiologically it competes with and replaces Ca^{2+} ions and accumulates in bones and all other tissues with high Ca^{2+} concentrations. Furthermore, oral administration of thallium (10 mg kg^{-1}) in mice resulted in rapid absorption and uniform distribution of thallium throughout the body. Since thallium (Tl^{+}) follows the biological movement of potassium (K^{+}), initially most of the Tl^{+} ions were excreted through urine and some of them accumulated in to the soft tissue. There is a greater accumulation of thallium in the tissues of the kidney and GI tract than in other tissues. The rapid absorption of thallium by these tissues may be due to the high blood perfusion rate but a relatively high removal of thallium should also be suggested (Fig. 17). The presence of thallium in liver, spleen and skeletal muscle was found to be intermediate although it is less than kidney and GI tract but more than myocardium and brain (Fig. 17). This indicates that in addition to blood perfusion, the potassium channel is also involved in the accumulation of thallium ions.

Spirulina powder significantly reduced the levels of strontium and thallium from various tissues. *Spirulina* is mostly insoluble in the GI tract and as such is passed out, it helps to bind to strontium or thallium and is excreted through faeces. This results in reduced absorption of strontium and thallium into the systemic circulation which ultimately prevents their accumulation in various organs or tissues of the body. Also the increased level of these metals in urine may be due to the chelating effect of the soluble fraction of *Spirulina*. The chelating property of *Spirulina* is unclear and is part of future systematic studies. The FTIR analysis of *Spirulina* powder in the present study revealed the presence of various metal binding functional groups. It has also been reported earlier that *Spirulina* powder contains a variety of functional groups such as -CO, -OH, COOH, -SH and -NH2 groups of various carbohydrates, amino acids and proteins (Chojnacka et al., 2005; Fang et al., 2011) that may play an important role in binding and removal of metal ions. Chojnacka et al. (2005) performed an experiment using methylene blue to determine the presence of active sites for the adsorption on surface of cells of *Spirulina*. Furthermore, Chojnacka et al. (2005) concluded that the main mechanism was ion exchange rather than adsorption due to the low adsorption surface and the presence of cations that appear in solution, and transfer from the biomass after biosorption. The low tissue retention and enhanced excretion of strontium and thallium may be due to the presence of metal binding functional groups on *Spirulina* (Schiewer and Wong, 2000; Aksu, 2002; Markai et al., 2003; Fang et al., 2011). These findings reveal that the presence of these anionic groups on the surface of *Spirulina* powder may be responsible for the formation of complexes with cationic metals and prevent their internalization. Therefore, the results of present study suggest that *Spirulina* treatment was most effective in reducing radionuclide burden from the body.

5. Conclusion

The results of the *in vitro* adsorption/binding studies of *Spirulina* for different metal ions showed that *Spirulina* exhibited excellent binding capacity for cobalt, strontium and thallium. *Spirulina* exhibited binding efficiency for barium and cesium at low extent compared to other metal ions. The binding capacity of *Spirulina* was higher for various metal ions at higher pH (SIF and water) compared to SGF. Langmuir and Freundlich adsorption isotherm models suggested that all the tested metal ions viz. cobalt, strontium, barium, cesium and thallium showed monolayer adsorption on *Spirulina* in water, SGF and SIF. Further *Spirulina* effectively reduced the whole body retention of strontium and thallium and enhanced its excretion through urine and faeces. Therefore, pulverized *Spirulina* can be used as efficient adsorbents to remove strontium and thallium from the body.

Declarations

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Availability of data and material: All data generated or analyzed during this study are included in this published article. A supplementary file is submitted along with the original article.

Author contribution statements: M.Y. and N.S. wrote the main manuscript text; M.Y., V.K. and S.J. collection of Data; M.Y. analysis of raw data and prepared all figures and tables; N.S. and M.K.C. supervision; M.K.C. editing the draft manuscript. All authors reviewed the manuscript.

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Figures

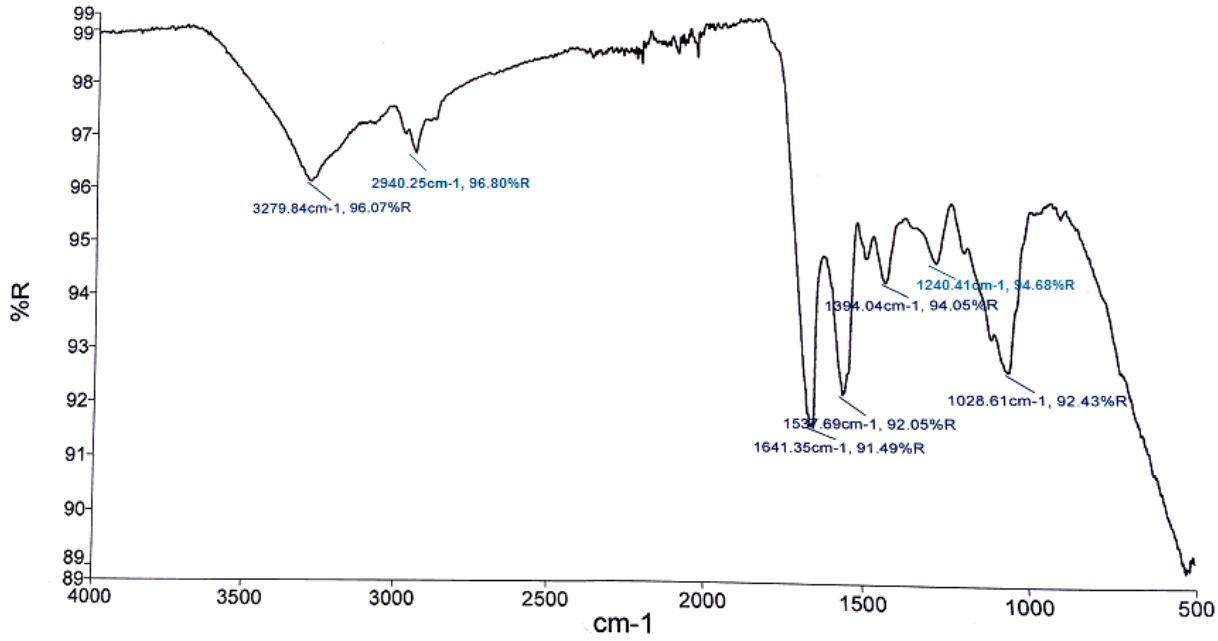

Figure 1

FTIR spectrum of *Spirulina* powder

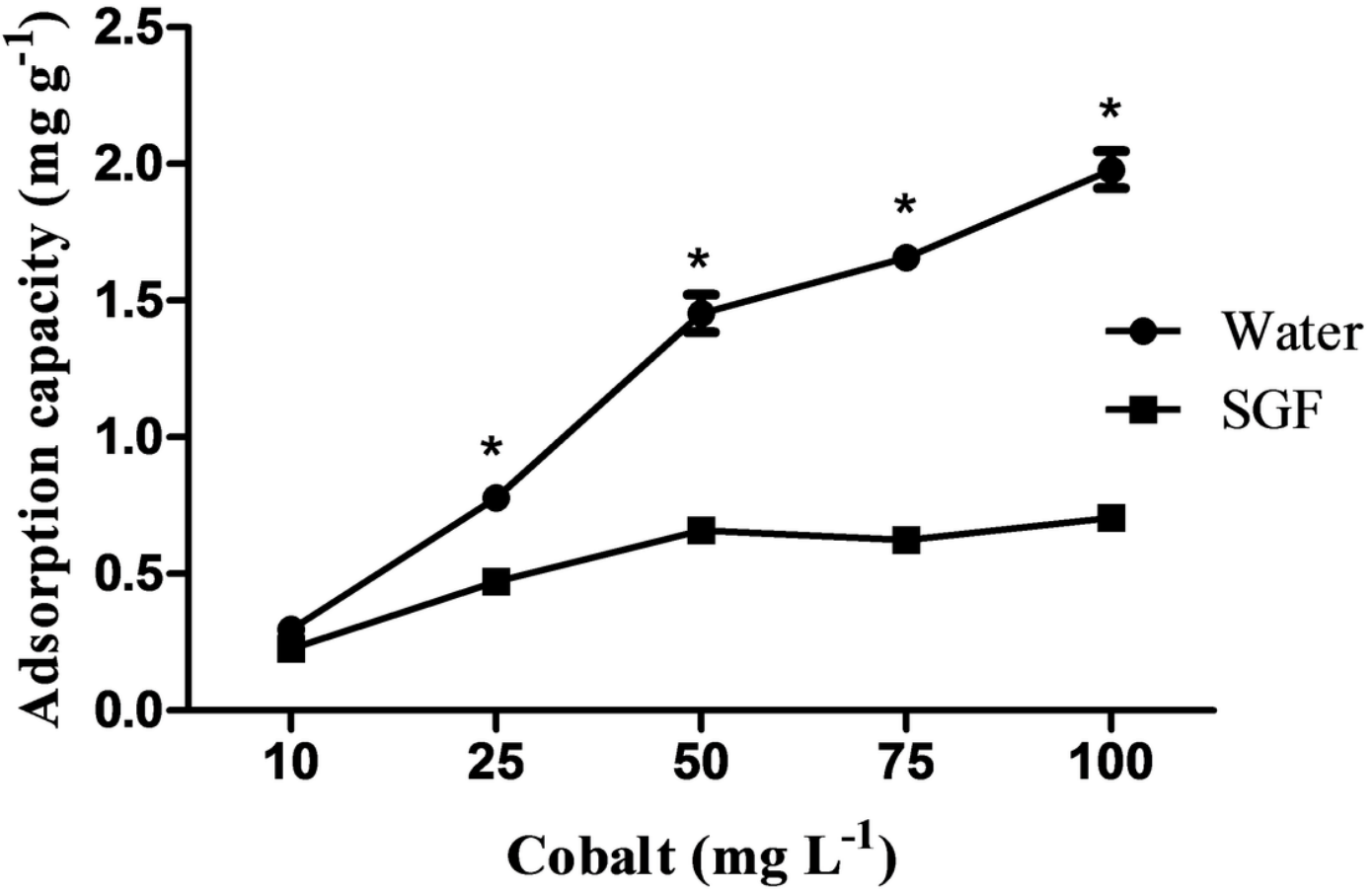

Figure 2

Effect of *Spirulina* on adsorption of cobalt in water and SGF. Adsorption capacity of *Spirulina* (10 g L^{-1}) at different concentration of cobalt (10-100 mg L^{-1}). Values are expressed in mean±SEM. (n=3). Significant value is *p< 0.001 when compared to SGF. (SGF- simulated gastric fluid).

Figure 3

Linearised Langmuir and Freundlich adsorption isotherm of *Spirulina* for cobalt. (A) Linearised Langmuir adsorption isotherm, **(B)** linearised Freundlich adsorption isotherm. (SGF- simulated gastric fluid).

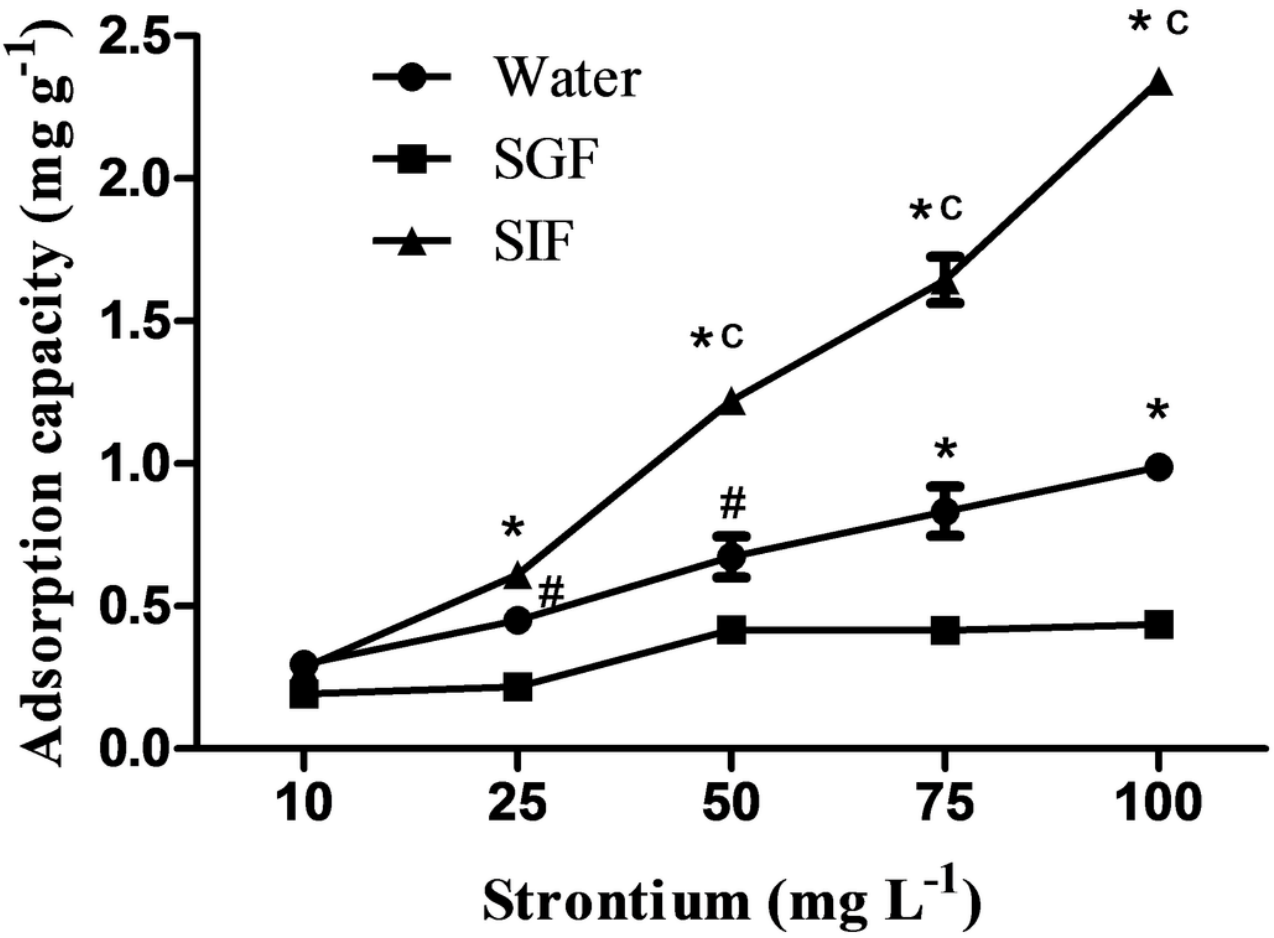

Figure 4

Effect of *Spirulina* on adsorption of strontium in water and simulated physiological solutions (SGF and SIF). Adsorption capacity of *Spirulina* (10 g L^{-1}) at different concentration of strontium (10-100 mg L^{-1}). Values are expressed in mean±SEM. (n=3). Significant values are $^{\#}p<0.01$ and $^{*}p< 0.001$ when compared to SGF and $^{c}p< 0.001$ when compared to water. (SGF- simulated gastric fluid, SIF- simulated intestinal fluid).

Figure 5

Linearised Langmuir and Freundlich adsorption isotherm of *Spirulina* for strontium. (A) Linearised Langmuir adsorption isotherm, **(B)** linearised Freundlich adsorption isotherm. (SGF- simulated gastric fluid; SIF- simulated intestinal fluid).

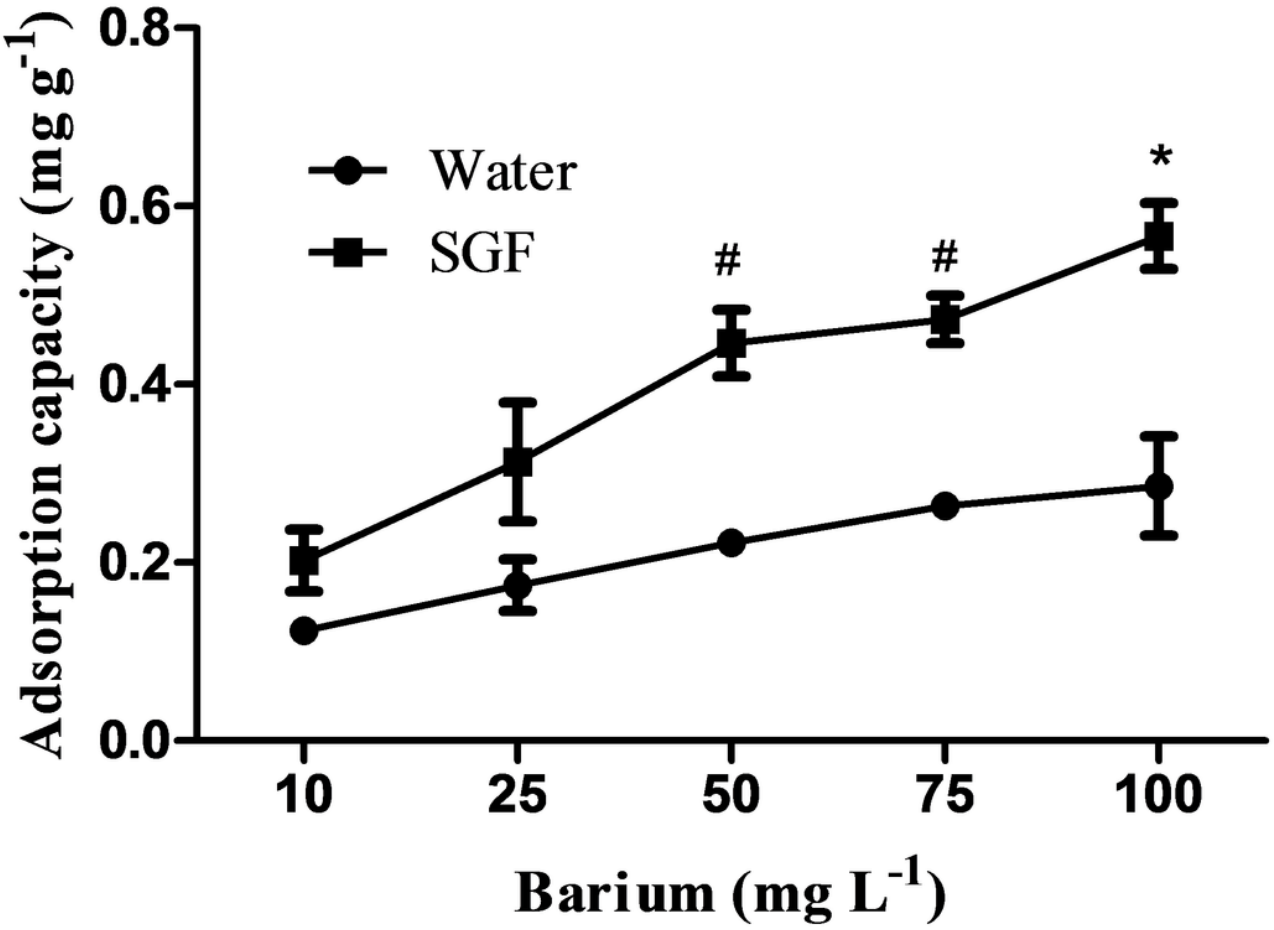

Figure 6

Effect of *Spirulina* on adsorption of barium in water and SGF. Adsorption capacity of *Spirulina* (10 g L^{-1}) at different concentration of barium (10-100 mg L^{-1}). Values are expressed in mean±SEM. (n=3). Significant values are $^{\#}p< 0.01$ and $^{*}p< 0.001$ when compared to water. (SGF- simulated gastric fluid).

Figure 7

Linearised Langmuir and Freundlich adsorption isotherm of *Spirulina* for Barium. (A) Linearised Langmuir adsorption isotherm, **(B)** linearised Freundlich adsorption isotherm. (SGF- simulated gastric fluid).

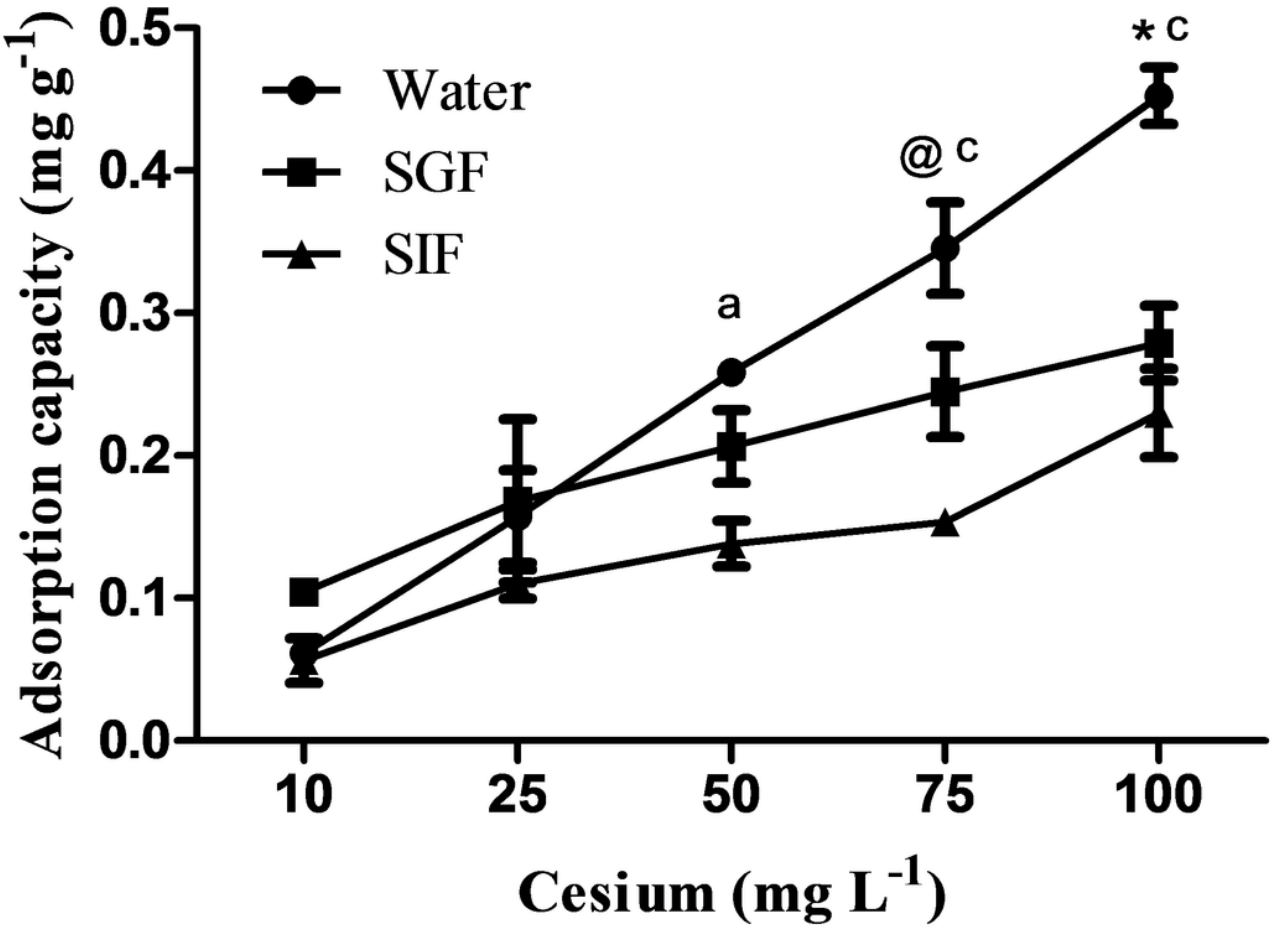

Figure 8

Effect of *Spirulina* on adsorption of cesium in water and simulated physiological solutions (SGF and SIF). Adsorption capacity of *Spirulina* (10 g L^{-1}) at different concentration of cesium (10-100 mg L^{-1}). Values are expressed in mean±SEM. (n=3). Significant values are $^{@}p< 0.05$ and $^{*}p< 0.001$ when compared to SGF, and $^{a}p< 0.05$ and $^{c}p< 0.001$ when compared to SIF. (SGF- simulated gastric fluid, SIF- simulated intestinal fluid).

Figure 9

Linearised Langmuir and Freundlich adsorption isotherm of *Spirulina* for cesium. (A) Linearised Langmuir adsorption isotherm, **(B)** linearised Freundlich adsorption isotherm. (SGF- simulated gastric fluid, SIF- simulated intestinal fluid).

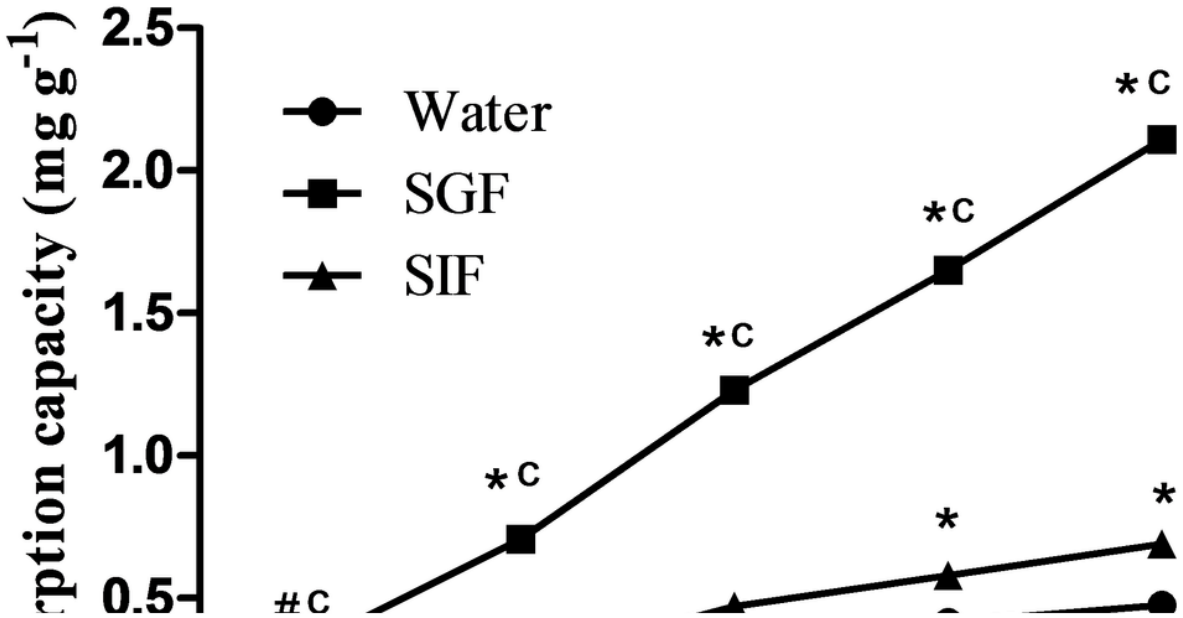

Figure 10

Effect of *Spirulina* on adsorption of thallium in water and simulated physiological solutions (SGF and SIF). Adsorption capacity of *Spirulina* (10 g L^{-1}) at different concentration of thallium (10-100 mg L^{-1}). Values are expressed in mean±SEM. (n=3). Significant values are $^{\#}p<0.01$ and $^{*}p< 0.001$ when compared to water, and $^{c}p< 0.001$ when compared to SIF. (SGF- simulated gastric fluid, SIF- simulated intestinal fluid).

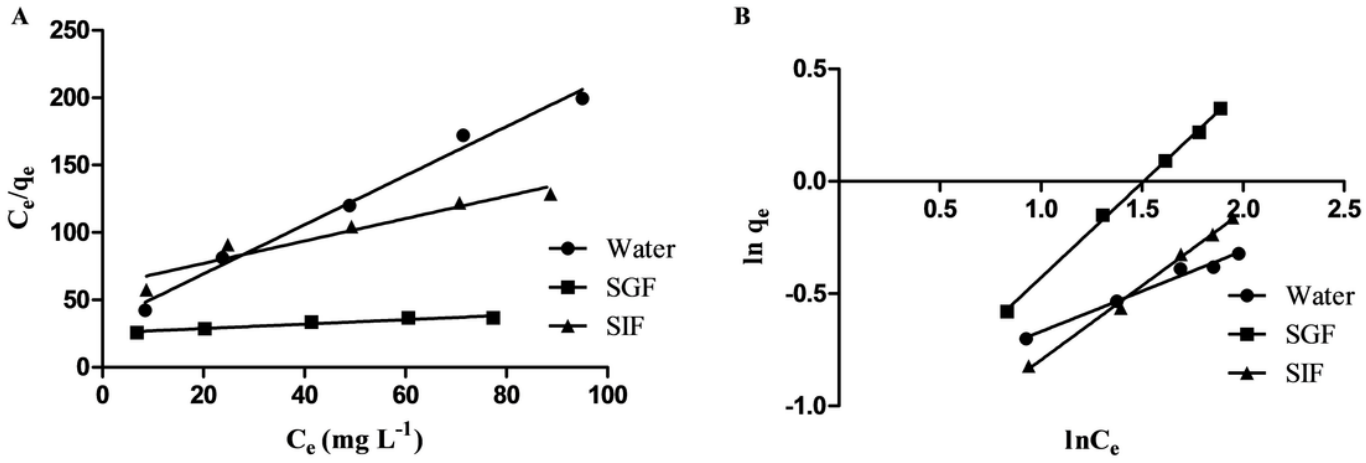

Figure 11

Linearised Langmuir and Freundlich adsorption isotherm of *Spirulina* for thallium. (A) Linearised Langmuir adsorption isotherm, **(B)** linearised Freundlich adsorption isotherm. **(**SGF- simulated gastric fluid, SIF- simulated intestinal fluid).

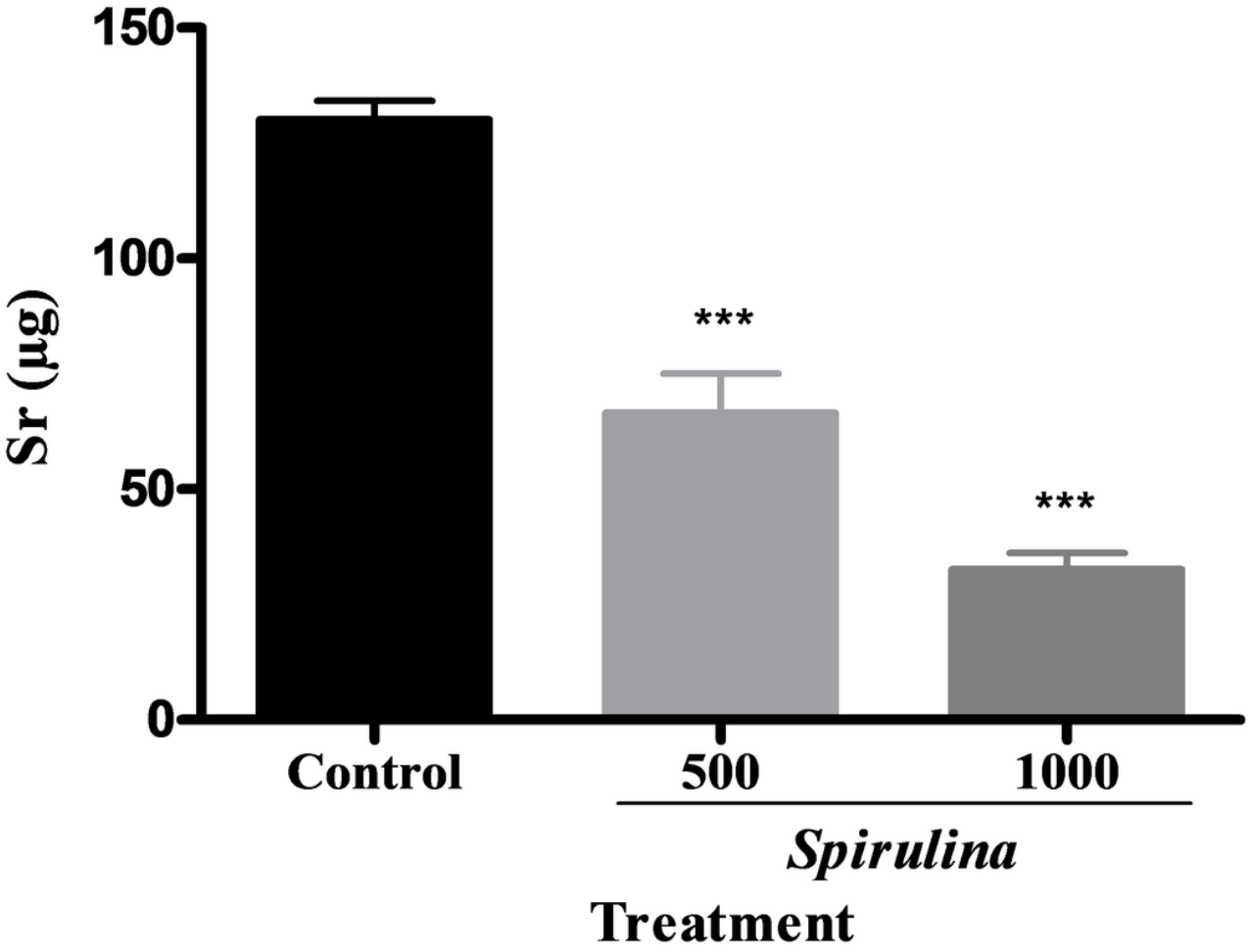

Figure 12

Effect of *Spirulina* on whole tissue retention of strontium. Values are expressed in mean±SEM (n=6). Significant values are *p< 0.05 and ***p< 0.001 when compared to control. The whole tissue retention in each animal was calculated as the sum of strontium levels (µg/g) in all collected tissues.

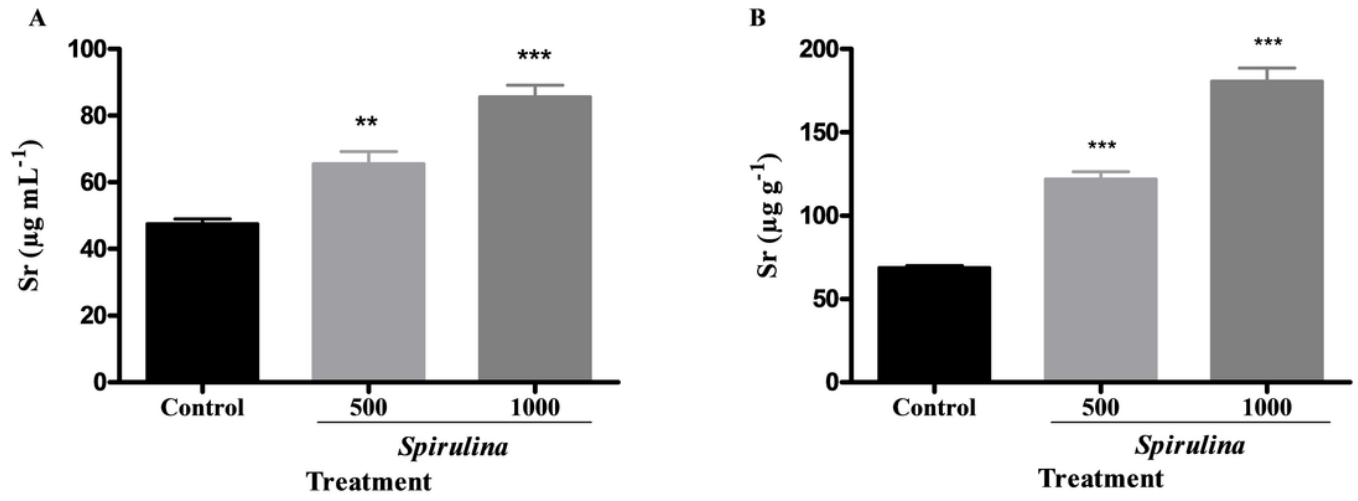

Figure 13

Effect of *Spirulina* excretion of strontium. (A) Excretion of strontium through urine and (B) excretion of strontium through faeces. Values are expressed in mean±SEM (n=6). Significant values are *p< 0.05 and ***p< 0.001 when compared to control.

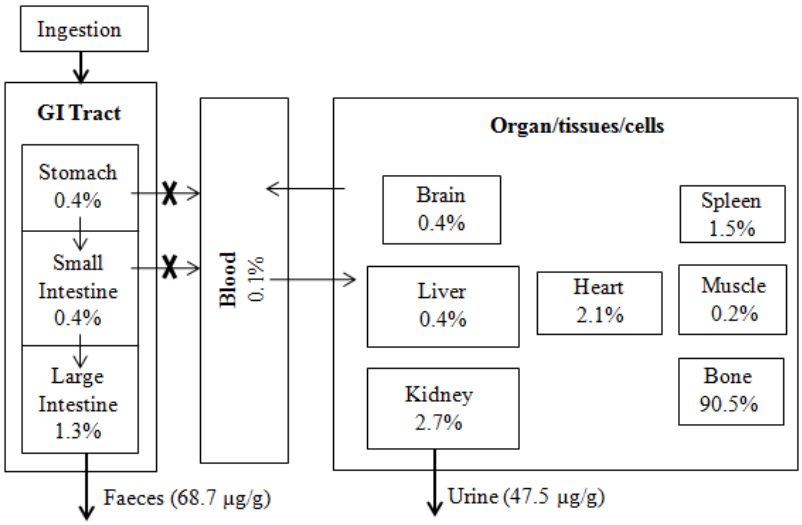

Figure 14

A schematic hypothesis for deposition and removal of strontium from body. The deposition of strontium is shown in percentage and excretion in actual value (µg g^{-1}). The dark arrow indicates movement of strontium with blood flow and dark bold arrows indicates ingestion (oral) and excretion (urine and faeces) of strontium. The bold cross indicates blockage of strontium internalization into the systemic circulation by *Spirulina* powder in gastrointestinal (GI) tract.

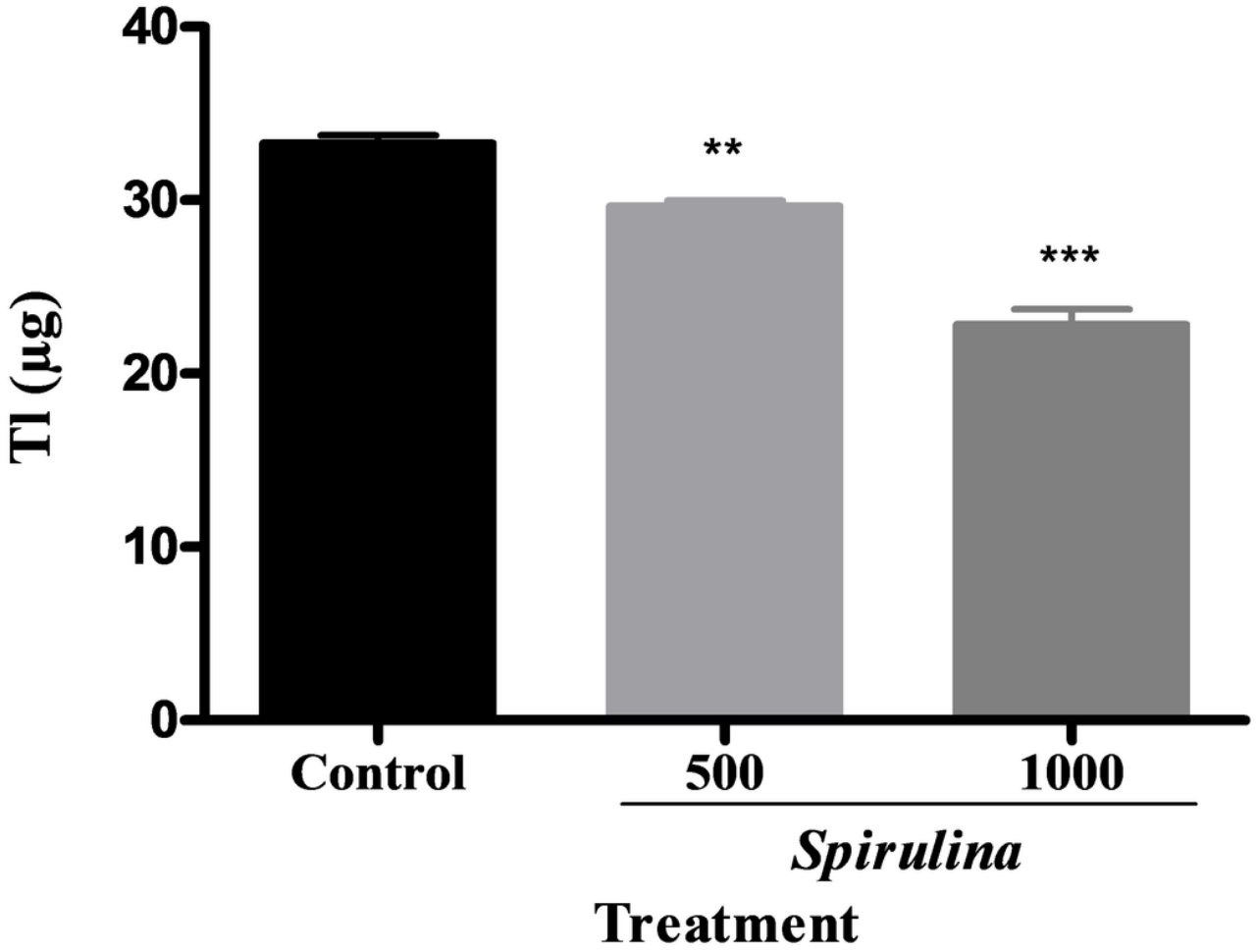

Figure 15

Effect of *Spirulina* on whole tissue retention of thallium. Values are expressed in mean±SEM (n=6). Significant values are ** $p < 0.01$ and *** $p < 0.001$ when compared to control. The whole tissue retention in each animal was calculated as the sum of thallium levels (µg/g) in all collected tissues.

Figure 16

Effect of *Spirulina* on excretion of thallium. (A) Excretion of thallium through urine and (C) excretion of thallium through faeces. Values are expressed in mean±SEM (n=6). Significant values are * $p < 0.05$ and *** $p < 0.001$ when compared to control.

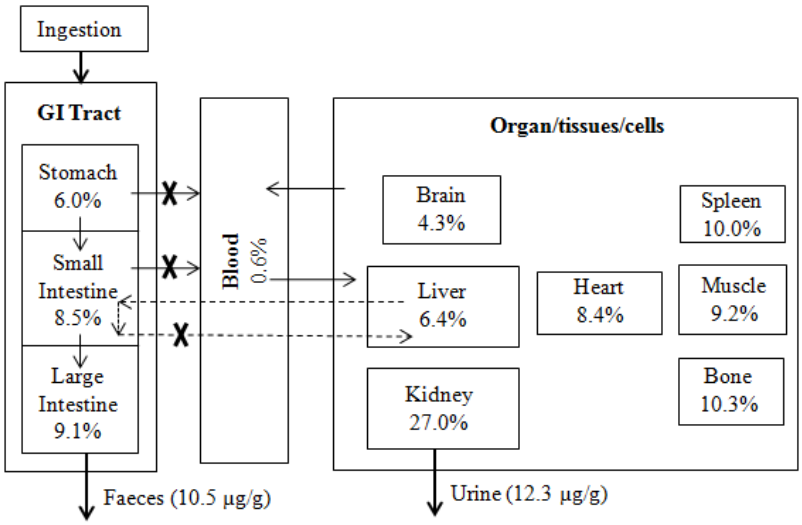

Figure 17

A schematic hypothesis for deposition and removal of Thallium from body. The deposition of thallium is shown in percentage and excretion in actual value (µg g^{-1}). The dark arrow indicates movement of thallium with blood flow, dotted arrow indicates movement of thallium through enterohepatic circulation and dark bold arrows indicates ingestion (oral) and excretion (urine and faeces) of thallium. The bold cross indicates blockage of thallium internalization into the systemic circulation by *Spirulina* powder in GI tract.

Supplementary Files

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