საქართველოს აგრარული უნივერსიტეტი

სადოქტორო სკოლა

სამეცნიერო მიმართულების კომისიის რეკომენდაცია

დისერტანტი: **ინგა თაბაგარი**

დისერტაციის სათაური:„ **სპილენძითა და ტყვიით დაბინძურებული წყლის გაწმენდის ეფექტური მეთოდის შემუშავება ლურჯ-მწვანე წყალმცენარის *Spirulina platensis* და ბიოსურფაქტანტების გამოყენებით“ Elaboration of the Effective Method of Water Cleansing, Polluted by Copper and Lead, to Using Algae *Spirulina Platensis* and Biosurfactants.“**

დისერტაციის დაცვის თარიღი: 25 თებერვალი 2020

დისერტაციის დაცვის კომისია:
რეცენზენტი 1: /ინგა აბდუშელიშვილი/
რეცენზენტი 2: /ელინა ბაქრაძე/
დაცის კომისიის თავმჯდომარე: /მიხეილ ასათიანი
დაცვის კომისიის წევრი: /თინათინ ბუთხუზი/
დაცვის კომისიის წევრი: /თამარ ვარაზი/
დაცვის კომისიის წევრი: /ლიანა შუბლაძე/
დაცვის კომისიის წევრი: /პიტერ ვონ ფრეგსტეინ ნიემსდორფი/
რეკომენდებულია დაცვისათვის სამეცნიერო მიმართულების კომისიის მიერ.

თავჯდომარე, /ცოტნე სამადაშვილი/ ___________________________

წევრი, /დავით ბედოშვილი/: ___________________________

წევრი, /ლევან გულუა/: ___________________________

წევრი, /თეო ურუშაძე/: ___________________________

წევრი, /ვლადიმერ ელისაშვილი/: ___________________________

წევრი, /ნატო კობახიძე/: ___________________________

წევრი /ია ფიფია/ ___________________________

სადოქტორო სკოლის კოორდინატორი: ___________________________ / ნატო კობახიძე/

თარიღი:

ინგა თაბაგარის

სადისერტაციო ნაშრომის

სპილენძითა და ტყვიით დაბინძურებული წყლის გაწმენდის ეფექტური მეთოდის შემუშავება ლურჯ-მწვანე წყალმცენარის *Spirulina platensis* და ბიოსურფაქტანტების გამოყენებით

ანოტაცია

წარმოდგენილი სამუშაო ფოკუსირებულია სპილენძითა და ტყვიით დაბინძურებული წყლის გაწმენდის ახალი მიდგომების შემუშავებაზე და დაფუძნებულია ლურჯ-მწვანე წყალმცენარე *Spirulina platensis* ეკოლოგიური პოტენციალის და მახელატირებელი აგენტების - ბიოსურფაქტანტების კომბინირებულ გამოყენებაზე.

კვლევის აქტუალურობა - წყლის სისტემების დაბინძურება მძიმე მეტალებით წარმოადგენს ერთ-ერთ ძირითად პრობლემას მრავალი ქვეყნისთვის და მათ შორის, საქართველოსთვისაც. დაბინძურების მთავარი წყარო კი ჩამდინარე წყლებია. უწყვეტი დაბინძურება განაპირობებს მეტალთა აკუმულაციას ცოცხალ სისტემებში და ადამიანის კვებით ჯაჭვში მოხვედრისას იწვევენ ჯანმრთელობის სერიოზულ პრობლემებს. წყლის გასუფთავების მეთოდები ძვირია და თან ყოველთვის არ არის ეკოლოგიურად უსაფრთხო. სწორედ ამიტომ, მძიმე მეტალებისგან წყლის გაწმენდის ალტერნატიული მეთოდების შემუშავება, რომელიც იქნება იაფი და ამავე დროს, დაფუძნებული გარემოსთვის უსაფრთხო, ბიოლოგიური ობიექტების გამოყენებაზე, ძალზე აქტუალურია.

ზემოაღნიშნულიდან გამომდინარე, ქიმიური დამაბინძურებლებისგან ჩამდინარე წყლების გაწმენდის ეფექტური მეთოდის შემუშავება და აგრარულ სფეროში უვნებელი წყლის გამოყენების შესაძლებლობა არის თანამედროვეობის აქტუალური საკითხი.

კვლევის სიახლე მდგომარეობს სპილენძისა და ტყვიის იონებით დაბინძურებული წყლის გასაწმენდად *Spirulina platensis* და ბიოსურფაქტანტების კომბინირებულ გამოყენებაში, აღნიშნული ლურჯ-მწვანე წყალმცენარეს რემედიაციული პოტენციალის გასაძლიერებლად. ჩემს მიერ ჩატარებული კვლევები წარმოადგენს სიახლეს და არასოდეს ყოფილა გამოკვლეული.

წარმოდგენილი ნაშრომი მიზნად ისახავს შეაფასოს *Spirulina platensis* – ის რემედიაციული შესაძლებლობები და გააძლიეროს მის მიერ მეტალთა შეთვისების პროცესი დამატებითი ფაქტორების გამოყენებით. ჩემი მიზანია გავაუმჯობესო სპირულინას მიერ მძიმე მეტალების ბიოსორბცია ნაკლებ დროში. შედეგად მიღებული სუფთა წყალი ემსახურება მდგრადი სოფლის მეურნეობისა და აგრარული სფეროს განვითარების მიზნებს.

სუფთა წყლით მომარაგება პირდაპირ პასუხობს მდგრადი განვითარების 17 მიზნიდან მე-6 მიზანს - „სუფთა წყალი და სანიტარია", რომელიც აერთიანებს ხუთ მნიშვნელოვან ინდიკატორს: 6.1 საყოველთაო ხელმისაწვდომობა სანიტარიასა და ჰიგიენაზე; 6.2 წყლის გამოყენების ეფექტურობის გაზრდა ყველა სექტორში; 6.3 წყალთან დაკავშირებული ეკოსისტემის დაცვა და შენარჩუნება; 6.4 წყლის ხარისხის გაუმჯობესება და დაბინძურების შემცირება; 6.5 სასმელი წყლის საყოველთაო ხელმისაწვდომობა. ასევე მჭიდრო კავშირშია მიზნებთან: მე-3 (ჯანმრთელობა და კეთილდღეობა), მე-11 (მდგრადი ქალაქები და დასახლებები), მე-12 (მდგრადი მოხმარება და წარმოება), მე-14 (წყლის ბინადრები) და მე-15 (დედამიწის ეკოსისტემები). მდგრადი განვითარების მიზნების პრიორიტეტულობაზე თანხმდება გაეროს ყველა წევრი ქვეყანა უკეთესი და უფრო მდგრადი მომავლის მისაღწევად 2030 წლისთვის.

სამუშაოს მთავარი ამოცანა - ისეთი ბიოსურფაქტანტის შერჩევა, რომელიც გააძლიერებს სპირულინას რემედიაციულ პოტენციალს, ასევე, სამიზნე დამაბინძურებლების იმ კონცენტრაციის პოვნა, რომელზეც სპირულინას ფიზიოლოგიური პარამეტრები შენარჩუნებული იქნებოდა.

სამეცნიერო ლიტერატურის მიმოხილვა - მოიცავს გარემოს დაბინძურების პრობლემის აქტუალობას, წყლის რესურსების დაბინძურების მიზეზებს, წყალში მძიმე მეტალების მოხვედრის გზებს, ინფორმაციას ფიტორემედიაციული მიდგომის უპირატესობებისა და შეზღუდვების შესახებ, სპირულინას ეკოლოგიური პოტენციალის შესახებ მომუშავე მეცნიერთა ნაშრომებისა და შედეგების შესახებ. ინფორმაციას ქიმიურ და ბიოლოგიურ ზედაპირულად აქტიურ ნივთიერებების და მათი თვისებებზე.

ბოლო 25 წელია აქტიურად მიმდინარეობს სპირულინას რემედიაციული უნარების გამოყენებაზე მუშაობა წყლის დასუფთავების მიზნით მსოფლიოს სხვადასხვა ქვეყანებში. მეცნიერებმა გამოიკვლიეს სხვადასხვა მეთოდი სპირულინას ბიორემედიაციული უნარების გასაუმჯობესებლად.

ლიტერატურულ მიმოხილვაში ყურადღება გამახვილებულია უახლეს მიღწევებზე სპირულინას რემედეაციული პოტენციალის გასაუმჯობესებლად. წარმოდგენილია ლიტერატურული წყაროები სპირულინაზე ნახშირწყლების დამატების შესახებ, მის მიერ აბსორბციული ლითონების რაოდენობის გაზრდის მიზნით (Markou et al. 2015). ასევე, შესწავლილ იქნა მშრალი და ნედლი სპირულინას მიერ მეტალთა შეთვისების უნარი ცალ-ცალკე და დადგინდა, რომ მშრალი ბიომასა უფრო ინტენსიურად ასუფთავებს მძიმე მეტალებით დაბინძურებულ წყალს, ვიდრე ნედლი (Al-Homaidan et al. 2014). თუმცა, ორივე კვლევაში დაფიქსირებული ზრდის მაჩვენებელი არ აღემატება 5%-ს. ჩემს მიერ ჩატარებული ექსპერიმენტების შედეგად კი სპირულინას მიერ აბსორბირებული სპილენძის რაოდენობა ტრეგალოზა ლიპიდის დამატების შედეგად გაიზარდა 73% -ით.

მეთოდოლოგია

კვლევის ძირითადი ობიექტი - ლურჯ-მწვანე წყალმცენარე *Spirulina platensis;*

კვლევის დამხმარე ობიექტი - ბიოსურფაქტანტები (რამნოლიპიდ1, რამნოლიპიდ2, ტრეგალოზალი).

დასაკვირვებლად შეირჩა ორი მძიმე მეტალისგან (სპილენძი და ტყვია) დაბინძურებული წყალი. კვლევები ჩატარდა ლაბორატორიულ პირობებში -დურმიშიძის სახელობის ბიოლოგიური ჟანგვის ლაბორატორიაში და აგრარული უნივერსიტეტის რემედიაციის ორანჟერეაში.

კვლევის მეთოდები - გრავიმეტრული, სპექტროფოტომეტრული, ატომურ-აბსორბციული სპექტროფოტომეტრული და ულტრასტრუქტურული.

მეტალთა რაოდენობრივი განსაზღვრა რემედიაციული პროცესის დინამიკაში ხდებოდა ორი მეთოდით - საინკუბაციო არეში ისაზღვრებოდა მეტალთა რაოდენობა სპექტრალურად, ხოლო სპირულინას ბიომასაში შეღწეული მეტალის კონცენტრაცია ისაზღვრებოდა ატომ-ადსორბციულ სპექტროფოტომეტრზე.

გარდა აღნიშნულისა კვლევის საწყის ეტაპზე სპირულინას უჯრედებში მეტალთა განსაზღვრისთვის გამოიყენებოდა ულტრასტრუქტურული კვლევის ელექტრონული მიკროსკოპიის მეთოდი.

ექსპერიმენტული ნაწილი მოიცავს 4 ძირითად ეტაპს:

პირველი ეტაპი - ეძღვნება სპირულინას კულტივაციას დიდი წარმადობით და ამისთვის შესაბამისი საკვები არეს შერჩევას. მძიმე მეტალების გავლენის შესწავლას სპირულინას ფიზიოლოგიურ აქტივობაზე (ბიომასისა და ქლოროფილის წარმოქმნაზე), სპილენძისა

და ტყვიის იონების სამუშაო კონცენტრაციის იდენტიფიკაციას შემდგომი ექსპერიმენტებისთვის.

მეორე ეტაპი - მოიცავს სპირულინას მიერ სპილენძის იონების შეთვისების დინამიკის შესწავლას მოდელურ ცდებში, სადაც გამოყენებულია სპილენძის იონებით ხელოვნურად დაბინძურებული წყალი.

სპირულინას უჯრედებში სპილენძის იონების შეღწევის ულტრასტრუქტურული განაწილების კვლევა ელექტრონული მიკროსკოპიის მეთოდით.

მეტალთა კონიუგაციაში მონაწილე ფერმენტის გლუტათიონ-S-ტრანსფერაზას აქტივობის გამოვლენა.

მესამე ეტაპი - მოიცავს სპირულინას მიერ ტყვიის იონების შეთვისების დინამიკის შესწავლას მოდელურ ცდებში, სადაც გამოყენებულია ტყვიის იონებით ხელოვნურად დაბინძურებული წყალი.

მეოთხე ეტაპი - მახელატირებელი აგენტის - EDTA-ს და ბიოსურფაქტანტების-რამნოლიპიდების და ტრეგალოზალიპიდის ეფექტის გამოკვლევა სპირულინას მიერ მძიმე მეტალების შეთვისების დინამიკაზე.

შედეგების გამოთვლა განხორციელდა Microsoft Excel-ში. სტატისტიკა ექვემდებარება ცალმხრივი დისპერსიის ანალიზს. სტატისტიკური ანალიზი გაკეთდა ANOVA-თი.

შედეგები:

- სპირულინას ბიომასა ზარუკას არეში 2 -ჯერ უკეთესად იზრდება, ვიდრე ფიზიოლოგიური ხსნარში;

- სპირულინას მიერ ქლოროფილის დაგროვების უნარი ფიზიოლოგიურ არეში 20% -ით დაბალია ვიდრე ზარუკას ხსნარში;
- Cu^{2+}-იონების 100 ppm კონცენტრაცია საინკუბაციო არეში, ბიომასის დაგროვების ინჰიბირებას 15%-ით, ხოლო ქლოროფილის შემცველობის შემცირებას 30%-ით ახდენს.
- სპირულინას ბიომასა ტყვიით დაბინძურებისას 48 საათის შემდეგ შემცირდა 18% -ით;
- ქლოროფილის წარმოება შემცირდა 23% -ით ტყვიით დაბინძურებულ წყალში 72 საათის შემდეგ.
- გლუტათიონის მაღალი კონცენტრაცია მნიშვნელოვნად ამცირებს თავისუფალი Cu^{2+}-ის შემცველობას საინკუბაციო ზონაში, რაც ნათლად მიუთითებს გლუტათიონ S- ტრანსფერაზას მონაწილეობაზე სპირულინას უჯრედების მიერ Cu^{2+} შეთვისების პროცესში;
- სპილენძის იონების 100ppm კონცენტრაციისას და საინკუბაციო არეში სპირულინას შემცველობისას - 4.5 გ/ლ, 72 საათის შემდეგ შთაინთქმება ხსნარში მყოფი სპილენძის 79 %.

- ტყვიის იონების 100ppm კონცენტრაციისას და საინკუბაციო არეში სპირულინას შემცველობისას - 4.5 გ/ლ 72 საათის შემდეგ შთაინთქმება ხსნარში მყოფი ტყვიის 85 %.
- სპირულინას უჯრედების ულტრასტრუქტურული კვლევა ნათლად აჩვენებს სპილენძის შეღწევას უჯრედში ინკუბაციიდან 24 საათის შემდეგ და იწყება მემბრანული სტრუქტურის რღვევა, ხოლო ინკუბაციის მეხუთე დღეს მეტალი სპირულინას ვაკუოლშია. (მეტალის კონცენტრაცია ხსნარში ამ ცდებში 50 ppm-ია);
- EDTA– მ სპირულინას მიერ შთანთქმული სპილენძის რაოდენობა 63%–ით გაზარდა;
- რამნოლიპიდ-1- მა (RL1) გაზარდა სპირულინას მიერ შთანთქმული სპილენძის რაოდენობა 42%–ით.

- რამნოლიპიდ-2- მა (RL2) გაზარდა სპირულინას მიერ შთანთქმული სპილენძის რაოდენობა 68%-ით;
- ტრეგალოზალიპიდმა (TRL) სპირულინას მიერ შთანთქმული სპილენძის რაოდენობა გაზარდა 73%-ით.
- RL1– მა შეამცირა სპირულინას მიერ შთანთქმული ტყვიის რაოდენობა 3 – ჯერ;
- RL2- მა შეამცირა სპირულინას მიერ შთანთქმული ტყვიის რაოდენობა 2.3 -ჯერ;
- TRL– მა შეამცირა სპირულინას მიერ შთანთქმული ტყვიის რაოდენობა 1.8 -ჯერ;
- ტყვიით დაბინძურებული წყლიდან, ბიოსურფაქტანტები მხოლოდ აჩქარებენ შთანთქმის პროცესს.
- საინტერესო ფაქტია ის, რომ სპირულინას უჯრედებში დაგროვილი მძიმე მეტალის იონები ბრუნდება წყალში ინკუბაციის მეოთხე დღეს.

მიღებული შედეგებიდან გამომდინარე, სპირულინას და ბიოსურფაქტანტების ერთობლივი მოქმედებით შესაძლებელი ხდება ტყვიისა და სპილენძის იონებისგან ჩამდინარე წყლების გაწმენდის იაფი და ეკოლოგიურად უსაფრთხო მეთოდის შემუშავება.

დასკვნები და რეკომენდაციები - წარმოდგენილი ნაშრომი განმარტავს ბიოსურფაქტანტების როლს სპირულინას რემედიაციული შესაძლებლობების გაზრდაში, ანუ სპირულინას სორბციული თვისებების გაუმჯობესებაში სპილენძისა და ტყვიის იონებით დაბინძურებულ არეში. მიღებული ექსპერიმენტული მონაცემები აჩვენებს მათი ერთობლივი მოქმედების ეფექტურობას.

მოცემული ექსპერიმენტული შედეგები შეიძლება გახდეს საფუძველი ფიტორემედიაციული ტექნოლოგიის შემუშავებისთვის, რომელიც დაეფუძნება *Arthrospira platensis*– ის გამოყენებას სამიზნე ტოქსიკანტებით დაბინძურებული წყლების გასაწმენდად. ამ ტექნოლოგიების მთავარი იდეაა Cu^{2+} და Pb^{2+} იონებით დაბინძურებულ

წყალში სპირულინას ბიომასის დამატება და პერიოდულად მისი მოცილება წყლის ზედაპირიდან (ან გაფილტვრა) რემედიაციული პროცესის დასრულებისთანავე.

აქვე უნდა იყოს გათვალისწინებული სპირულინას ბიომასის მიერ მეტალთა იონების შთანთქმის ეფექტური დრო და ის ნიუანსიც, რომ მეტალთა იონები სპირულინას უჯრედების გაჯერების შემდეგ (მეოთხე დღეს) ბრუნდება უკან, საინკუბაციო ხსნარში, სწორედ ამ მიზეზის გამო, სარემედიაციო რეზერვუარში მეტალთა შემცველი სითხის დაყოვნება უნდა მოხდეს მხოლოდ 2-3 დღით, რაც სპირულინას ეფექტური შთანთქმის მაქსიმუმს ემთხვევა.

ტყვიით დაბინძურებული წყლიდან მეტალების მოცილების პროცესი ბიოსურფაქტანტების გამოყენებით დაჩქარებულია 24 საათით, რაც გათვალისწინებული უნდა იყოს ჩამდინარე წყლების გამწმენდი ტექნოლოგიის სწრაფი რეაგირების სტრატეგიის შექმნისას.

შემდგომი კვლევები გულისხმობს სპირულინას ულტრასტრუქტურულ ანალიზს, რაც გამოავლენს სპირულინას მიერ მეტალების სორბცია - დესორბციის მექანიზმებს უფრო ზუსტად.

დისერტაციის შემდგომი განვითარებისა და გამოყენების პერსპექტივა:
ამ ნაშრომს აქვს პოტენციალი სამომავლო კვლევების განვითარებისა, რათა უზრუნველყოს ყველაზე ეფექტური და რესურსების დამზოგველი წყლის რემედიაციული მიდგომა.

მეცნიერული თვალსაზრისით - დისერტაციის ექსპერიმენტული კვლევების საფუძველზე შექმნილია მძიმე მეტალებით ჩამდინარე წყლების გასუფთავებისთვის მეცნიერულად დასაბუთებული სწრაფი სტრატეგია.

ტექნიკური თვალსაზრისით - მომავალი კვლევებით დადგინდება მძიმე მეტალებით დაბინძურებული ჩამდინარე წყლების რემედიაციის ტექნოლოგიური რეგლამენტი და შეიქმნება ბიოტექნოლოგიური სქემა. მიღებული ექსპერიმენტული მონაცემების ინტერპრეტაციით შესაძლებელი გახდება სპილენძისა და ტყვიის იონებისგან ჩამდინარე წყლების გაწმენდის მეთოდის პილოტური გამოცდა.

კომერციული თვალსაზრისით - მოხდება შემუშავებული ბიოტექნოლოგიისა და სამეცნიერო იდეების პატენტირება. ტექნოლოგიის პილოტური ტესტირება და დაინტერესებული ორგანიზაციებისთვის კონკრეტული კომერციული რეკომენდაციების გავრცელება.

დისერტაციის ექსპერიმენტული მასალები გამოქვეყნებულია მაღალი რეიტინგის საერთაშორისო-სამეცნიერო ჟურნალებში 3 სტატიის სახით:

Tabagari I, Kurashvili M, Varazi T, Adamia G, Gigolashvili G, Pruidze M, Chokheli L, Khatisashvili G, Niemsdorff PVF (2019) *Application of Arthrospira (Spirulina) platensis against chemical pollution of water*. **Journal Water**, MDPI 11:1759-1768. https://doi.org/10.3390/w11091759

Tabagari I, Chokheli L, Adamia G, Kurashvili M, Varazi T, Pruidze M, Khatisashvili G, Niemsdorff PVF (2020) *The effectiveness of Arthrospira platensis for the purification of copper-contaminated water*. Water, Air, and Soil Pollution, **Springer,** 231:470-478. https://doi.org/10.1007/s11270-020-04841-2

Tabagari I, Tamar Varazi, Liana Chokheli, Maritsa Kurashvili , Marina Pruidze , Gia Khatisashvili , Olena Karpenko, Vira Lubenets, Peter von Fragstein und Niemsdorff (2021) *Enhancement of Spirulina platensis remediation action using biosurfactants for wastewater treatment*. International Journal of Environmental Research, **Springer**. In press.

Elaboration of the Effective Method of Water Cleansing, Polluted by Copper and Lead, to Using Algae *Spirulina Platensis* and Biosurfactants

Inga Tabagari

Dissertation has been submitted to the Council of Agrarian Sciences of the Agrarian University of Georgia to obtain the academic degree of Doctor of Agrarian Sciences

Scientific Supervisors:

- Tamar Varazi, Full Professor
- Peter von Fragstein und Niemsdorff, Professor Dr. Dr. h.c. mult.
- Liana Shubladze, PhD.

Agricultural University of Georgia

Tbilisi 2021

Author's declaration

As the author of the submitted doctoral dissertation, I declare that my dissertation is an original work and that materials published, or defended by other authors in it are used in accordance with the proper citation rules.

Inga Tabagari

December 2021

Abstract

Pollution of water systems with heavy metals is an urgent problem for many countries. Wastewater is the main pathway for pollution of rivers and water systems from heavy metals. Modern human activity is closely related to the use of various chemical compounds. Their distribution in the environment occurs via different pathways. Toxics like boomerangs are returning to humans. This problem is especially acute in developing countries. Water treatment is expensive and not always ecologically friendly. That is why alternative methods of water purification based on the use of harmless biological objects are very relevant. Ensuing from the aforementioned, elaboration of methods of environmental cleansing from chemical contaminants and the possibility of using harmless water in the agricultural field is a topical issue of modernity. Based on this study, recommendations can be made to the agricultural sector on the use of safe water in both food and irrigation technology.

This thesis focused on the framework of a new approach of water cleansing polluted by copper and lead, by using combined action of the ecological potential of algae *Spirulina platensis* and chelating agent – biosurfactant.

This thesis aims to estimate remedial possibilities of *Spirulina platensis* and enhancing of metal uptake processes by using additional tool.

The main idea is to find the concentration of target pollutants at which will safe the physiological parameters of Spirulina as a remediator, also choose biosurfactant, which enhance the remediation properties of algae.

It has been chosen to work the concentration of metals – 100 ppm at which *Spirulina platensis* during 72 Hours uptake 79 % of Cu^{2+} ions and 85 % of Pb^{2+} ions from incubation medium.

It is clear from a series of tests that the adsorption of Cu^{2+} and Pb^{2+} ions by biomass of Spirulina is improved and sped up by using ecologically friendly biosurfactants – Rhamnolipids and Trehalose lipid.

Specifically, the process of metals removal from polluted water is sped up by 24 hours, which will be considered in creating a quick response strategy of wastewater treatment technology.

Particularly, Rhamnolipid-1 enhances the absorption of copper by Spirulina by about 42%, Rhamolipid-2-by 68%, and Trehalose lipid by 73%. Moreover, their priority compared to the toxic EDTA itself is obvious.

Obtained experimental data will possible to provide an inexpensive and ecologically friendly approach for purifying wastewater from copper and lead ions.

Keywords: 1. *Spirulina platensis*; 2. wastewater, 3. lead, 4. copper, 5. chelating agents.

biosurfactants.

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Abbreviation

AAS.. Atomic absorption spectroscopy
Cu. .... Copper
EDTA .... Ethylenediaminetetraacetic acid
et al. .... and others
EPA .... The Environmental Protection Agency
g. .... Gramm
GSH .... Glutathione S-transferase
HLPW. .... High Level Panel on Water outcome documents
h.m.. Heavy metal
L .... Liter
LCD. .... Liquid Crystal Display
MEPAG .... Ministry of Environment Protection and Agriculture of Georgia
Pb .... Lead
ppm .... parts per million
RL1 .... Rhamnolipid1
RL2 .... Rhamnolipid2
Sp. .... Spirulina
SAFS .... Sustainable Agriculture and Food Systems
TRL .... Trehalose Lipid
UNESCO .... The United Nations Educational, Scientific and Cultural Organization
UV .... ultraviolet light

1. Introduction

Nowadays, water contamination is a widely recognized challenge. Continuous heavy metal pollution is a serious threat to all forms of life in the environment due to its toxic effects. These metals are highly reactive at low concentrations and can accumulate in the food web, causing serious public health problems.

As in other developing countries, the problem of polluted wastewater in Georgia remains one of the main problems. Recently, there has been an increase in oncology in the regions, next to open pits. Water contaminated with heavy metals used for irrigation causes disease in the population (MEPAG 2019). Functioning of polymetallic factories are considerable harming the ecological systems in the environment. Operation of RMG Gold and Copper Mine in the SE part of Georgia causes severe ecological problems in the region.This is a deplorable situation in the Bolnisi municipality. The monitoring was conducted of heavy metals in system "water-soil-plant" in the area of Kazreti (Madneuli) – villages: Balichi, Ratevani, Naxiduri, Xidiskhuri. According to the results obtained in the soil, content of heavy metals are significantly higher than the allowable concentration limit, as well as in Rivers Kazretula and Mashavera (Avkopashvili et al. 2017; Avkopashvili et al. 2020). Based on the villages studied in Chiatura (Khreiti and Rgani), it was found that the incidence of oncological diseases in the local population is directly proportional to the location of open quarries (distance from the settlement) and the duration of their development (Kvarackhelia et al. 2017).

Goal of the work is to clean water, polluted with heavy metals – Copper and Lead, by inexpensive methods, which will also be environmentally friendly. This thesis has the potential to provide the most cost-effective and resource-saving approach to water recovery in order to avoid its contamination with various hazardous chemicals.

Our research object and tool for water remediation is Algae Spirulina. This cyanobacterium is actively used to clean the soil from organic pollutants that remain abundant in agricultural lands.

Spirulina platensis has great potential for absorbing organic pollutants. Also, due to the ability of aged Spirulina to rise to the surface of the water, it is easy to remove and provide model testing in reservoirs. Therefore, we hypothesized that Spirulina would also be a purifier of water contaminated with heavy metals. Our purpose is to prove the efficacy of Spirulina in the treatment of heavy metal polluted water and enhance this ability.

The structure of the dissertation is as follows:

Literary review - consisting of an overview of the problem of environmental pollution, the causes of pollution of water resources, the reasons for the provision of heavy metals in water, information on the success and limitation of phytoremediation, on the ecological potential of Spirulina and information on chemical and biological surfactants and their properties.

Research methods - all analytical methods used in the experiments are given

Experimental part - consists of 4 main stages.

First stage - is the cultivation of Spirulina with a large biomass and the selection of an appropriate nutrient medium. Study of the influence of heavy metals on the physiological activity of Spirulina (formation of biomass and chlorophyll), identification of the working concentration of copper and lead ions for further experiments.

Second Stage – Investigation of Copper ions uptake processes by *Spirulina platensis* from artificially polluted water.

Third Stage - Investigation of Lead ions uptake processes by *Spirulina platensis* from artificially polluted water.

Last Stage – Investigation of chelating agent- EDTA and Biosurfactants – Rhamnolipids and Trehalose lipid influence on heavy metals uptake dynamics.

The unique nature of results of the work consists in the proposed method of waste water cleansing, uniting ecological potential of Algae *Spirulina platensis* and Biosurfactants, which provide speed up and enhancing adsorption of metals in Algae biomass and create quick strategy for creation water remediation biotechnology. It's worthy of mentioning that cleansing of water area via the proposed method is ecologically absolutely harmless.

How the dissertation results will be developed and used in further expiring researches:

- From scientific point of view – elaboration of scientifically based quick strategy of heavy metals containing waste water treatment.

- From technical point of view – establish of regalements for wastewater cleansing biotechnology

- From commercial point of view – To create principles of patent of elaborated biotechnology and scientific ideas. Provide field in situ testing of the technology and Dissemination of concrete commercial recommendations to interested organizations.

2. Literature Review

2.1. Environmental pollution - problem of the mankind

Human activity is steadily connected with production and application of different chemical compounds. Annually, hundreds of million tons of chemicals are produced all over the world (Dincer and Zamifirescu 2014). These compounds also products received owing to their abiotic and biotic transformation are often of toxic nature. They are distributed in different ways. They are concentrated in tremendous amounts in the biosphere and effect significantly the ecological balance. The results of urbanization, development of industry and transport, production of chemicals for agriculture, military and other activities are harmful and toxic compounds like boomerang revert to human (Ali et al.2019).

The environment is sensitive to changes in human behavior and continuous development in the use of new technologies. New challenges to human life and natural resources need overcome as urban populations are expected to grow in the coming decades. Nowadays global chemical pollution of the ecosystem's biosphere, waters, and soil tops the hazardous level. Due to official data for the beginning of XXI century owing to anthropogenic activities the total polluted area consists a 10% of the whole land area (Prokacheva and Usachev 2002). Ensuing from afore mentioned, elaboration of methods of environmental cleansing from chemical contaminants is the key ecological problem of the mankind.

2.2. The natural water clearing on waterworks and remediation of numerous toxic precipitates

The sources of water pollution are urban, agricultural, and industrial wastewater. According to a recent study, estimates that 380 billion m^3 (m^3 = 1000 L) of wastewater is generated annually in the world, which is five times the volume of water passing through Niagara Falls annually (Qadir et al. 2020). Heavy metals occur in ecosystems from extended development of ore deposits. It makes grave problems with environmental protection issues (Frieman et al. 1999; Rasheed et al. 2019). Industrialization has always seemed to be the key to wealth and a better life, but in fact it has been shown that while it leads to better living conditions in certain respects, it affects the environment and ultimately contributes to a worse ecosystem (Mgbemene et al. 2016). In every country, with the exception of highly developed ones, wastewater flows straight into the environment without any treatments that cause danger to human health, crop quality, soil fertility, and the whole ecosystem (Cho 2011). Integrated urban water management (IUWM) addresses issues that 80% of wastewater is still discharged untreated and helps cities' progress to a circular economy (Kim 2018). Currently urban environmental pollution comprises hundreds of substances, including aliphatic, aromatic and polycyclic hydrocarbons, phenols, pesticides, organochlorine compounds, explosives based on nitro compounds, heavy metals, radionuclides (Kvesitadze 2006).

Proceeding from the above mentioned, the creation of quick response strategic approaches against chemical pollution of numerous precipitates of sewage sludge will resolve the important environmental problem. Low-quality water damages crop production and dangerous our food which is harmful to human life (Khan and Ghouri 2012). Adequate and clean drinking water is necessary to ensure human health. Human activities increase the risk of water pollution which directly leads to a reduction in biodiversity and socio-economic development (Kim 2018). Air, water and soil are contaminated with various metals due to anthropogenic activities that disrupt the normal biogeochemical cycle. Biodiversity is widely used by both developed and developing countries to remove metals from the environment (Prasad 2003).

General sources of contaminated water are urban and manufactured runoff, i.e. agricultural sewage (Potters 2013). Most of the industries, such as the electroplating, automotive, battery manufacture, microelectronics, metallurgical, etc. use metals, mostly copper, which contribute to the growth of toxicants in the water bodies. They pile up in photosynthetic organisms and move contaminants to consumers, including humans (Celekli et al. 2010). Almost 90% of wastewater in developing countries is flowing into rivers, seas, and lakes without any treatment. Demographic processes affect the availability, quality, demands, and consumption of water (UNESCO 2009).

Since the 18th century, human activity bounded with the Industrial Revolution has caused a change in the composition of the atmosphere (Brekke et al. 2009). There is evidence that due to human-induced work global climate is changing (UNESCO 2009). Industrial wastes are either connected directly to streams or other natural bodies of water or discharged into municipal sewers, and their characteristics vary widely depending on the source of production and the raw materials used in the industry. Industrial wastewater treatment can be carried out partially or completely using biological processes (Muralikrishna and Manickam 2017).

Toxic Compounds formed at the mass water treatment using the coagulative-flocculated processes constitute special ecological problems. At present, the fresh river and lake water of industrial countries, which one mainly used to produce a tap water for large cities, contains numerous pollutants, ranging from traditional natural humic substances to contaminations of an anthropogenic origin such as toxic organic substances (phenols, petroleum products, pesticides, herbicides etc.), heavy metals and even radionuclides with a long half-life (such as ^{137}Cs and ^{90}Sr in Ukraine after the Chernobyl accident). Additionally, the situation with a surface water quality deteriorates year by year. The most convenient and common method of the mass water treatment on waterworks in many countries is the process of coagulative-flocculated water clearing. It includes the treatment of water firstly with coagulant (aluminum sulfate, aluminum oxychloride, ferrite or other metal salt) and then with flocculant (polymeric substance of organic or inorganic origin), which precipitates all connected substances. The resulting sediments which contain high concentrations of toxic organic substances, heavy metals and other pollutants are ejected into so-called "silt fields", thus making a significant threat to the environment. The improvement of the coagulative-flocculated process of water clearing through the search for increasingly effective flocculants, capable of complex water purification from pollutants as natural as anthropogenic origin, is promising tool to improve a tap water quality in large cities. At the same time, the creation of new biotechnologies for remediation of numerous precipitates of sewage sludge will solve an important environmental problem (Denchak 2018).

2.3. Heavy metals existing in ecosystems

Occurrence of heavy metals in environment connected with prolonged exploitation of ore deposits. This creates seriously problems connected with the question of environmental protection. These problems are especially sharp when development of deposits occur by open excavation using quarry method. Quarry management, dressing industrial complex, safe warehousing and handy disposal of "tails" and overburden rocks are most difficult technological processes, demanding the capable management with complete maintenance of rules and regulations provided by country's legislation regarding to environment protection. Otherwise the gross violation of natural ecological equilibrium and processes of contamination of life importance ecosystems – ground and surface waters, soils, atmospheric air, vegetation (including cultivated verdure) get irreversible character, which reflects directly on the condition of health of local population (Ali et al. 2019).

Nowadays, heavy metal pollution of terrestrial and above-water ecosystems has become a global environmental challenge. The gross contravention of natural ecological equilibrium and ecosystems get a permanent reaction, reflecting on the local population's health. Some metals affect biological function and growth, while other metals accumulate in one or more organs, causing many serious diseases such as cancer. The pharmacokinetics and toxicological processes in humans are described for each metal (Briffa et al. 2020). Environmental pollution is one of the most serious global problems. Rapid industrialization, the discovery, and development of new oil fields, modern agricultural methods are contributing significant amounts of toxic heavy metals to the environment. Heavy metals are some of the most apparent pollutants in soil, water, and air. Heavy metal pollution is constantly increasing, causing serious toxic effects on all forms of living organisms and altering the properties of the soil and its biological activity. Excessive accumulation of heavy metals in plant foods can have serious negative consequences for food quality and safety. Consuming such foods poses a potential risk to global food security and healthy lifestyles (Tumanyan et al. 2020). Heavy metal toxicity has been proven to be a serious threat and there are several health risks associated with it. The toxicity of metals depends on the absorbed dose, the route of exposure and the duration of exposure, i.e. acute or chronic. Accumulation in the body can affect to people in a variety of ways. Often affects development of children (Jaishankar et al. 2014).

Environmental problems have always attracted great attention from scientists. Toxic pollution is a serious environmental problem that poses a serious threat to human health and agricultural production. Heavy metals and pesticides top the list of environmental toxins that threaten nature. Heavy metals, lead (Pb) and copper (Cu) have a negative impact on the agricultural ecosystem (plants and soil) and human health. Heavy metals and pesticide residues accumulate in soils and plants, causing various human diseases (Alengebawy et al. 2021; Zischka 2014).

The mobilization of heavy metals by humans through extraction from ores and processing for various purposes has resulted in the release of these elements into the environment. This pollution poses a threat to the environment and human health. Some heavy metals are carcinogenic, mutagenic and endocrine disruptors, while others cause neurological and behavioral changes, especially in children (Hazrat et al. 2013). The cause for 3.1% of deaths is the unhygienic and poor quality of water (Qadri et al. 2019). 10% of residents consume poisoned food and herbs that irrigated by contaminated water. Almost 90% of sewage water flows into rivers and seas without treatment in most developing countries. Currently, there is no global monitoring of wastewater management, which is a prerequisite for better health development (Eid 2015).

Excess copper causes physiological and proteomic changes in plants. Copper is an essential trace mineral for plants. Copper, present in high concentrations in soil, is also considered a major toxic to plant cells due to its potential inhibitory effect on many physiological and biochemical processes. The interference of heavy metals in proteins associated with sprouting has not been well documented at the proteomic level. Much research indicates a good correlation between physiological and biochemical changes in germinating rice seeds exposed to excess copper (Ahsan et al. 2006). In the human body, copper accumulates in the liver, which leads to liver failure and metabolic disorders – Wilson's disease. It causes skin pigmentation, low energy, and chronic fatigue. An enormous amount of copper, as well as lead, causes the most severe complications of the human nervous system (Thomson 2006).

The existence of heavy metals in foods is a prompt issue, involving pollution of the food chain and damage to population health. Contamination of foods by heavy metals has a number of various sources. The most considerable ones are poisoning of the soil from which foods are produced; remaining sludge; chemical compost and pesticides used in cultivation. In specific, fish is the product most impactful of heavy metal pollution, because heavy metals are accumulated in the food chain. The hazard of heavy metals is especially acute because they are not chemically or biologically degradable. Once emitted into the environment, they can continue to exist for hundreds of years (Masindi and Muedi 2018).

Due to World Health Organization, Lead is a cumulative toxicant that affects multiple body systems and is harmful to young children. Lead in the body is distributed to the brain, liver, kidney, and bones. It is stored in the teeth and bones, where it accumulates (WHO 2019).

2.4. The health impact of outdoor environmental pollution

Environmental Pollution became apparent in global burden of disease. Exposures to environmental pollution remain a major source of health risk throughout the world, though risks are generally higher in developing countries, where poverty, lack of investment in modern technology and weak environmental legislation combine to cause high pollution levels. Associations between environmental pollution and health outcome are, however, complex and often poorly characterized. Levels of exposure, for example, are often uncertain or unknown as a result of the lack of detailed monitoring and inevitable variations within any population group. Exposures may occur via a range of pathways and exposure processes. Individual pollutants may be implicated in a wide range of health effects, whereas few diseases are directly attributable to single pollutants. Long latency times, the effects of cumulative exposures, and multiple exposures to different pollutants which might act synergistically all create difficulties in unravelling associations between environmental pollution and health. Nevertheless, in recent years, several attempts have been made to assess the global burden of disease as a result of environmental pollution. Chronic diseases, especially cancer, cardiovascular disease, and respiratory diseases, are the leading causes of morbidity and mortality worldwide (Zischka 2014).

More recently, ambient air pollution has been implicated in increasing the incidence and mortality from lung cancer and from cardio-pulmonary diseases. Present-day urban air pollution comprises hundreds of substances, including Sulphur dioxide, ozone, nitrogen oxide, nitrogen dioxide, carbon monoxide, carbon dioxide, particulate matter, rubber dust, polycyclic aromatic hydrocarbons, and many different volatile organic compounds. Despite the major efforts that have been made over recent years to clean up the environment, pollution remains a major problem and poses continuing risks to health. The problems are undoubtedly greatest in the developing world, where traditional sources of pollution such as industrial emissions, poor sanitation, inadequate waste management, contaminated water supplies and exposures to indoor air pollution from biomass fuels affect large numbers of people. Even in developed countries, however, environmental pollution persists, most especially amongst poorer sectors of society. In recent decades, too, a wide range of modern pollutants have emerged—not least, those associated with road traffic and the use of modern chemicals in the home, in food, for water treatment and for pest control. Most of these pollutants are rarely present in excessively large concentrations, so effects on health are usually far from immediate or obvious. Detecting small effects against a background of variability in exposure and human susceptibility, and measurement error, poses severe scientific challenges. The progressively larger number of people exposed to environmental pollution nevertheless means that even small increases in relative risk can add up to major public health concerns. As the impact of human activities and issues of environmental health become increasingly global in scale and extent, the need to recognize and to address the health risks associated with environmental pollution becomes even more urgent. Effective action, however, requires an understanding not only of the magnitude of the problem, but also its causes and underlying processes, for only then can intervention be targeted at where it is most needed and likely to have greatest effect (Chen and Goldberg 2009).

2.5. Phytoremediation - successful and ecologically friendly technology

Phytoremediation in comparison with other nonbiological and biological technologies has great advantage. It is envisaged by many private or state companies while projecting and accomplishing remediation or prevention activities. This advantage is revealed in several aspects: Phytoremediation can be successfully applied in any type of chemical pollution. Plants and microorganisms are capable to assimilate and detoxify wide spectrum of organic and inorganic toxicants: aliphatic, cyclic and polycyclic hydrocarbons, phenols, polychlorinated biphenyls, organochlorine solvents, pesticides, explosive compounds, heavy metals, radionuclides etc.

Phytoremediation is universal not only for chemical compounds, but also for the objects needing cleansing, because plant can absorb toxic compounds from the three elements of the biosphere- soil, water and air.

Phytoremediation is accomplished directly in polluted site *in situ* which allows to maximally maintain soil characteristics (structure, rhyzosphere content, humidity, porosity etc). Other remediation technologies are carried out basically under *ex situ* – soil needs excavation, transporting, enloding into special bioreactors or displacement in spots etc. Obviously, such processing of soil doesn't lead to its absolute restoration, because these processes significantly damage soil structure that needs several tens of years to be formed. Besides, each operation needs extra expenses, which significantly raises cost price of technology.

Phytoremediation is more economic and profitable in comparison with other technologies. It is estimated that phytoremediation saves 4-fold expenses needed for cleansing one and the same object than other technologies. Economic effect can be much higher in some cases. In the special literature cases are described when application of phytoremediation technologies for cleansing of the environment polluted with heavy metals and radionuclides costs 1000-fold cheaper (EPA 2001).

Phytoremediation at the same time implies remediation and preventive inspection, because it allows not only to restore chemically polluted environment but to limit for long period of time or absolutely avoid dissemination of toxic compounds from hotbeds of pollution. Besides, purposeful planting of greenery protects the soil from exhaustion and erosion.

Here should be pointed shortcomings of phytoremediation – for achievement of the goal via phytoremediation months and even years are needed, with application of other methods provides environmental cleansing within several days or weeks. This shortcoming is revealed especially in case of heavy metals. For instance, if mercury concentration in the polluted soil is 2500mg/kg, than at application of such technology as phytoextraction, soil total remediation can be achieved within ten years. Very high concentrations of pollutants e.g. at oil accidental pouring, may significantly reduce greenery growth. Therefore, toxic compounds are more rapidly disseminated from the pollution hotbeds into the environment (e. g. groundwater), than plants and microorganisms provide their assimilation.

Although application of phytoremediation as technological approach of removal of chemical contamination of the environment started at the end of XX century, there are several hundreds of cases of its purposeful exploitation. Via this method were purified soils and waters polluted by: heavy metals, radionuclides, oil products, aromatic and polycyclic hydrocarbons, pesticides, explosives, chlorinated solvents etc. the striking example of successful application of phytoremediation technology is the activity of Phytotech, the company which cleaned the soil contaminated with Uranium in Ohio (USA), and water reservoirs after Chernobyl accident (Schnoor 1997).

Approaching the reclamation of contaminated sites, traditional remediation technologies working on the ex-situ scale are considered not the best choice for both ecological and economic reasons, due to the requirement to threat very large number of polluted substrates as a waste. These limitations have moved the interest of scientists and policy makers to more sustainable technologies as phytoremediation. Phytoremediation relies on the properties of plants and the associated microorganisms to absorb accumulate and/or degrade contaminants while tolerating them. To be more effective, phytoremediation requires the availability of plant species and microorganisms' strains in which these metabolic processes are naturally enhanced.

2.6. *Spirulina platensis* as a tool for bioremediation

Cleaning up a polluted environment using remedial skills is a priority in the modern world. Bioremediation is an attractive and successful treatment method for removing toxic waste from a polluted environment. Bioremediation is actively involved in the degradation, eradication, immobilization or detoxification of various chemical wastes and physical hazardous materials from the environment through the overarching action of microorganisms. The main principle is the decomposition and transformation of pollutants into less toxic forms. Bioremediation can be carried out ex-situ and in-situ, depending on several factors, including but not limited to cost, site characteristics, type and concentration of contaminants. Bioremediation is the most efficient, economical and environmentally friendly tool for managing polluted environments (Sharma 2020). Since heavy metals are not biodegradable, they build up in the environment and subsequently contaminate the food chain. Thus, the elimination of heavy metal contamination deserves due consideration. Molecular mechanisms are used to completely appreciate the devices of metal uptake, and translocation in plants. phytoremediation is the best solution to the problem. Phytoremediation is the use of plants and associated soil microbes to reduce the concentration or toxic effects of pollutants in the environment. It is a relatively new technology that is perceived to be cost-effective, efficient, new, environmentally friendly and popular technology (Hazrat et al. 2013).

Particular attention is paid to bioaccumulation, mechanism of action, and transport of heavy metals. Meanwhile, synergistic and antagonistic interactions between heavy metals and pesticides and their combined toxic effects have been discussed (Alengebawy et al. 2021; Zischka 2014).

Recovery using conventional physical and chemical methods is uneconomical and generates large volumes of chemical waste. The bioremediation of hazardous metals has generated considerable and growing interest over the years. The use of microbial biosorbents is environmentally friendly and cost-effective, hence, it is an effective alternative for remediation of heavy metal contaminated environments. Microbes have different metal binding mechanisms, which are more capable of biosorption of metals. The purpose of microbial biosorption is the removal and/or recovery of metals and metalloids from solutions using living or dead biomass and their components (Ayangbenro and Babalola 2010). Biosorption is a promising technology that aims to create new, cheap (inexpensive), and highly efficient materials for use in wastewater treatment technology. Algae belong to a diverse group that can contribute to important industries. Their main application is the production of a wide range of primary and secondary metabolites used in the food, pharmaceutical, and cosmetic industries. Many articles have collected information from published works for the last dissertation, which discuss the alternative use of various algae (micro and macro) in raw or modified form as promising biosorbents for the disinfection of water or wastewater. many important factors affecting the adsorption process, such as the influence of the pH of the solution, contact time, temperature, and dose of the adsorbent (Anastopoulos and Kyzas 2015).

Since the early 2000s, researchers from many countries involved purify wastewater from heavy metals using different algae (Daud et al.2018; Lin et al. 2020; Romera et al. 2007; Sekomo et al. 2012; Verma and Suthar 2015; Zeraatkar et al. 2016). Some researchers are considering the microbial transformation of heavy metals, the interaction of microbes with metals, and various approaches to the microbial rehabilitation of heavy metals from water bodies. state that bacteria and fungi are effective microbes that often transform heavy metals and eliminate toxicity (Chaturvedi et al. 2015; *Kim et al. 2015).* Because of testing, three different strains of *Enterobacter* *cloacae* can absorb almost 0.03% of heavy metal from a copper-contaminated environment. *Micrococcus luteus* DE2008 is considered a microorganism capable of regenerating lead (sorption 0.7%) and copper (sorption 0.5%) of contaminated environments (Puyen et al. 2012).

During the past 10 years, many studies have shown that Spirulina purifies water contaminated with chemical pollutants. A recent study in a river (Yamuna) clarifies the ability of algae to remove heavy metals (Cu, Cd, Ni, Cr, Pb) from water (Kumar et al. 2020). Heavy metals affect produce of Spirulina biomass. Copper affects microorganisms. It Disrupts cellular function and inhibits enzyme activities (Dixit et al. 2015; Nagajyoti et al. 2010; Salem et al. 2000).

Modern phytoremediation technologies may include different methodological approaches, depending on their purpose. One of the methods of cleaning the chemically polluted waters is Phycoremediation, based on application of algae. There are some examples of using algae for cleaning water, polluted with heavy metals (Phang et al. 2015). Plants that excessively accumulate metals are ideal model organisms and attract the attention of scientists around the world due to the fact that they are used in phytoremediation technology. Metallic hyperaccumulators have the ability to overcome basic physiological problems. The potential of hyperaccumulators for use in phytoremediation depends on their growth rate (i.e., biomass production) and the rate of metal accumulation (g of metal per kg of plant tissue). The two main reasons limiting the global application of this technology are the slow growth rates exhibited by most natural metal hyperaccumulators (Prasad 2003).

It is necessary to maintain the technology with maximum efficiency and low-cost investment, acceptable for a wide range of metal contamination (Verma and Suthar 2015). Therefore, an obvious need for technology with optimal efficiency and lower investment, which can be a reasonable solution for treating water with a wide range of heavy metal contamination. Recently published studies show that ion exchange and membrane filtration are the most frequently studied and widely used for the treatment of wastewater contaminated with metals. Ion exchange made it possible to completely remove Cd (II), Cr (III), Cu (II), Ni (II), and Zn (II) with an initial concentration of 100 mg / L, respectively. It is important to note that the total cost of treating metal-contaminated water varies depending on the process used and local conditions. Overall, technical feasibility, ease of installation, and cost-effectiveness are key factors in choosing the most suitable treatment for inorganic effluents (Kurniawan et al. 2006). Bioremediation is a flexible technology based on the unique ability of organisms to cleanse any contaminated environment. The advantages are technological feasibility, low costs, minimal rainfall, and competitive performance (Kurashvili et al. 2018).

Cyanobacteria are the most suitable bio sorbents and bio accumulators because of their wide distribution and flexible metabolism. They are the best detoxifiers. The processes of biosorption and bioaccumulation depend on natural metabolic processes when toxic ions are absorbed instead of basic ions (Cepoi et al. 2016). The bioadsorption potential of *Arthrospira* species, namely A. *indica*, A. *maxima*, and A. *platensis*, has been tested for toxicity of lead, chromium, and cadmium under laboratory conditions, and it has been shown that A. *platensis* is the most potential candidate for the bioadsorption of lead, chromium, and cadmium (Balaji et al. 2014). Many researchers have proven that the removal of heavy metals from wastewater can be carried out by bacteria, yeast and fungi. Among them, Spirulina algae are relatively cheap and are especially capable of accumulate metals. Due to their high absorption capacity, they mainly work for the disposal of heavy metals. In particular, *Arthrospira platensis* produces well in salted water or seawater, even in an alkaline environment (Kumar et al. 2020; Palaniswamy and Veluchamy 2017). It has well known Spirulina as beneficial to human health for a long time, as it releases heavy metals from the liver, gastrointestinal tract, and reproductive system. *Arthrospira platensis* has promising effects on improving heavy metal toxicity, which is mainly attributed to its intrinsic antioxidant activity (Bhattacharya 2020).

Spirulina which is at the present time extensively used in food production and pharmacology (Khan et al. 2005), can be used also as a phytoremediation agent for cleansing the chemically polluted waters. The cells of blue-green Algae contain huge amounts of compounds (peptides, amino acids, enzymes and etc.) able to connect as organic pollutants, so ions of heavy metals. The unique ability of cyanobacteria to bind to the absorption of heavy metals has been going on for 25 years (Fang et al. 2011). Biosorption can be determine as the capacity of biological materials to scratch up heavy metals from sewage via the process of absorbtion through physicochemical pathways or metabolism. In some studies, the economically important microalga (cyanobacterium) Spirulina *platensis* has been used as a biosorbent to remove copper from aqueous solutions. Cyanobacteria were exposed to various concentrations of copper, and copper adsorption by biomass was evaluated under various conditions, including pH, contact time, temperature, adsorbate concentration, and dry biomass concentration. Increased copper adsorption by non-living biomass was recorded with a gradual increase in pH, and the maximum absorption by biomass was observed at pH 7 (Al-Homaidan et al. 2013). The absorption of metals is a complex process that depends on several factors, including the pH of the solution, ionic strength and temperature. The surface of the cell wall of biomass is characterized by several functional groups, such as carboxyl, phosphate, hydroxide, amide / amine groups, etc., have a great influence, since biomass macromolecules - proteins, lipids, carbohydrates, significantly affect the absorption of metals (Markou et al. 2015).

An attempt of modern science is aimed at cleaning wastewater from heavy metals using cheap and environmentally friendly means. A prime example of this is the use of tea waste which removes copper and lead ions from wastewater. The investigator claims that tea waste is the cheapest compared to other alternative remedies (Amarasinghe and Williams 2007), However, Spirulina is a cyanobacteria that, unlike the tea growing season, does not need a whole year, but multiplies very quickly and is highly productive (Neves et al. 2019).

Recently, researchers (in Italy, Belgium, Holland, Ukraine, India) actively involved in the purification of the most important characteristics of wastewater from heavy metals and various synthetic wastes using many types of algae (Sekomo et al. 2012; Verma and Suthar 2015). The possibility of using Spirulina is described in the study by scientists from Durmishidze Laboratory at the Georgian Agrarian University, who studied the effects of different concentrations of DDT on Spirulina growth capacity and the intensity of DDT uptake by Spirulina. In parallel, the penetration of a toxic substance into Spirulina cells and its effect on cell ultrastructure were observed using the electron microscopic method. DDT was found to easily penetrate Spirulina into cells and cause partial destruction of organics. After 15 days, Spirulina removes about 70% of DDT from contaminated water (Kurashvili et al. 2018).

2.7. Chelating agents to enhance the remediation potential of *Spirulina platensis*

Observe on influence of chelators on the ability of Spirulina we have started with EDTA as a classic cheating agent. The absorption of metals by plants using chelates has only recently been discovered in the reduction industry. The simultaneous accumulation of lead, arsenic, copper and cadmium in plants after the introduction of chelating agents into the soil is a promising improvement in phytoremediation technology. One of the most powerful and commonly used chelating agents is ethylenediaminetetraacetic acid (EDTA), which forms a complex with many metal contaminants in the natural environment. The addition of EDTA to treatment systems increased the uptake of heavy metals by plants, which is especially noticeable in the case of copper and lead(Dipu et al. 2012). Chelating agents are able to bind to metal ions to form complex structures that are easily removed from the intracellular or extracellular space (Swaran and Pachauri 2010). We have determined the absorbed quantity of heavy metals by atomic absorption spectroscopy. As a result, – add EDTA to the environment contaminated with copper increased the absorption capacity of the *Arthrospira platensis*, and with lead, on the contrary, decreased it. The chelating agent EDTA (ethylenediaminetetraacetic acid) is a compound that is widely used throughout the world in everyday life and industry, being one of the anthropogenic compounds with the highest concentrations in inland waters of Europe. This review describes the use of EDTA and its behavior after release to the environment. Degradation of EDTA has been achieved under laboratory conditions; however, in vivo studies show poor biodegradability. It is concluded that EDTA behaves as a persistent substance in the environment and that its contribution to the bioavailability and remobilization of heavy metals in the environment is of serious concern (Oviedo and Rodriguez 2003). Complexes of heavy metals with EDTA are much more tenacious and not biodegradable (Grčman et al. 2001).

Having outcomes of EDTA, we continued our research on biosurfactants. These compounds have been considered a “green” product with renewable resources (Elis acirc ngela et al. 2015). In the era of global industrialization, natural resource exploration has served as a source of experimentation for science and advanced technology, leading to the production of high aggregate value products in the global marketplace, such as biosurfactants. The production of biosurfactants is considered one of the key development technologies in the 21st century. Biodegradability and low toxicity have led to the intensification of scientific research on a wide range of industrial applications of biosurfactants in the field of bioremediation (Santos et al. 2016). In nature, biosurfactants are amphiphilic molecules capable of emulsifying and reducing the surface tension of water, mainly glycolipids are the best known and studied, produced by Pseudomonas aeruginosa. These compounds are produced by microorganisms (bacteria, actinomycetes and fungi) that have genes, metabolic pathways and, as a result, are physiologically adapted to absorb insoluble organic matter. Recently, interest in its biotechnological production has increased due to their environmental, industrial, pharmaceutical applications, etc. Through the study of microbial biodiversity in a terrestrial or aquatic environment contaminated with persistent organic compounds that are not soluble in water, microorganisms with tensoactive properties can be isolated and biodegradable pollutants. This can be done using selective culture media, strain gage tests, and molecular instruments. Biosurfactants are promising products (Ocampo 2016).

Macromolecules are important cellular components of biological systems responsible for a large number of functions necessary for the growth and stability of living organisms. A large number of macromolecules are present in mixtures with surfactants, where a combination of hydrophobic and electrostatic interactions is responsible for the specific properties of any solution. It has been demonstrated that surfactants can promote the formation of helices in some proteins, thereby contributing to the formation of the protein structure. On the other hand, there is extensive research on the use of surfactants for the solubilization of drugs and pharmaceuticals; therefore, it is clear that the interaction between surfactants with macromolecules is important for many applications, including environmental processes and the pharmaceutical industry. Surfactants are amphiphilic molecules able to reduce the surface tension between two unmixable phases (Aguirre-Ramirez et al. 2021; Otzen 2017). Many bacteria create biosurfactants during the grown-on water substrates. Biosurfactants are forceful under extreme temperature, pH, and saltiness (Kumar and Das, 2018). The biosurfactants' ability to increase cell membrane permeability helps enhance various biologically active preparations (Lubenets et al. 2013). Most overall, biosurfactants are glycolipids. Microbial glycolipids are four fundamental groups: Rhamnolipids, Trehalose lipids, Sophorolipids, and Mannosylerythritol lipids. To form a better view of the influence of biosurfactants, we examined three kinds of biosurfactants – Rhamnolipid 1 (RL1) and Rhamnolipid (RL2), and Trehalose lipid (TRL). As the name suggests, Rhamnolipids contain a Rhamnose unit or units linked to a 3-hydroxyl fatty acid unit or units across the β-glycosidic bond. Rhamnolipids are mono-Rhamnolipids and di-Rhamnolipids, depending on the number of Rhamnose units in the molecule (Irorere et al. 2017; Tiso et al. 2017). Recently investigated the use of biosurfactants rhamnolipids in soil and groundwater treatment to remove petroleum hydrocarbons and pollutants containing heavy metals. there are current recovery technologies with the integration of rhamnolipid and the effects and mechanisms of rhamnolipid to promote the removal of pollutants for these technologies are discussed. rhamnolipid-based methods for remediation of heavy metal contaminated sites were presented and discussed (Liu et al. 2017). Rhamnolipids weaken the outer shell of the microorganism, promote and accelerate the penetration of heavy metals (Smyth et al. 2010). These surfactants are economically useful because they can be obtained from a variety of inexpensive substrates such as carbohydrates, vegetable oils and even industrial waste, which offers good potential for commercial use. By controlling environmental factors and growth conditions, high yields of rhamnolipids can be achieved. Rhamnolipids have good physicochemical properties in terms of surface activity, stability and emulsification activity (Patel and Desai 2007; Pornsunthorntawee et al. 2010). Trehalose lipids and Rhamnolipids can speed up biomass production (Koretska et al. 2020), so we hypothesize that because of this ability, the amount of metal absorbed will improve after they are added. These ability of Rhamnolipids and Trehalose lipids has inspired us to raise Spirulina capacity to absorb copper ions. We evaluated the consequences, and all three biosurfactants had different effects on *Arthrospira platensis* capabilities. As a result, TRL worked best.

2.8. Sustainable Development Goals

The topic of the doctoral theme is related to relevant and modern environmental issues and can provide useful advice for the private sector and farmers. Water embedded in almost all the SDGs, particularly those dealing with food, energy and the environment. Water is the gossamer that links the web of the 17 SDGs and their 169 targets (Ait-Kadi, 2016).

2.8.1. SDG6 (Clean water and sanitation)

Increasing global attention over water resources is one of the main challenges in 2015 by the World Economic Forum (Ait-Kadi 2016). A unt of household wastewater without cleaning flows into the river. It contains toxicants, solid waste, and bacterial contaminants (Haseena et al. 2017) Responsibility for contamination of surface and groundwater belongs in manufacturing plants. Pollution depends on the type of production. Toxic compounds are one of the main problems of diminished water quality. 25% cause of harmful contamination by industrial activities. Great concern following environmental pollutants in Georgia - heavy metals (copper, manganese, lead, arsenic, etc.), pesticides (among them to obsolete pesticides), oil hydrocarbons, Polycyclic aromatic hydrocarbons, etc. (MEPAG 2019). This factor was decisive in choosing my topic because clean water is direct which plays a crucial role in sustainable agriculture development.

2.8.2. SDG3 (Good health and well-being)

Adequate and clean drinking water is necessary to ensure human health. Human activities increase the risk of water pollution which directly leads to a reduction in biodiversity and socio-economic development (HLPW 2018). The cause for 3.1% of deaths is the unhygienic and poor quality of water (Haseena et al. 2017). 10% of residents consume poisoned food and herbs that irrigated by contaminated water. Almost 90% of sewage water flows into rivers and seas without treatment in most developing countries. Currently, there is no global monitoring of wastewater management, which is a prerequisite for better health development (Eid 2015).

Polluted soil and water position serious exogenous and human health problems that can be partly solved use modern phytomedicine technology. Cost-Effective access based on the capacity of a plant — the ability to turn the concentration of toxicants in the environment and the potential to metabolize molecules in their tissues — is the principal factor promoting to the solution of this actual issue (EPA 2001).

The drinking water condition in the world has improved. However, the situation is still worrying and the challenge of the Millennium Development Goals of 2012 is to increase water quality in the next three years (Eid 2015). Despite this the quality of water in Georgia needs to be improved because the result is already reflected on the health of the population (Japaridze 2004) so our research is aimed at precisely this.

2.8.3. SDG11 (Sustainable cities and communities)

Cities' water consumption will increase from 15–20% to 30% at the same time the supply of fresh water will remain unchanged. Cities make a significant contribution to water quality. Globally, 80% of wastewater is still not treated (HLPW 2018).

My topic is aimed at the development of sustainable cities and communities because it can prevent progress polluted water.

2.8.4. SDG14 (Life below water)

Proper wastewater management is a prerequisite for environmental health (Eid 2015). Water ecosystems - lakes, wetlands, and streams are particularly sensitive to water quantity and quality. Precipitation, nutrients and toxic substances formed from the water stream from the ocean, river or lakes enter these ecosystems (WWDR 2015).

The rivers of Georgia polluted by heavy metals due to open quarries. Part of the population feeds with fish. Mentioned toxicants are a threat to the underwater residence that affects human health (Bakradze 2014). In Bolnisi region environmental contamination is caused by gold and copper mining. Uncontrolled manufactured waste water flowing into the Kazretula River causes to the death of living organisms (Avkopashvili et al. 2017).

2.8.5. SDG15 (life on land)

The aquatic ecosystem provides a habitat for many plant and animal classes (WWDR 2015). Wastewater in Georgia flows directly into rivers that threaten both animals and harvest since these waters used for irrigation (Bakradze 2014). For my research, I choose Algae Spirulina and chelating agent – Biosurfactant Both of them are harmless and cannot bring any dangers for life on land. Increase phytoremediation efficiency of plants as the best tool for water cleansing (Plocinickaz 2011).

2.8.6. SDG16 (Peace, justice and strong institutions)

Powerful organizations with protective justice are most important for saving people from any disease nascent from polluted water (HLPW 2018). Effective decisions are directly reflected in the socio-economic sector because the water-related strategy is affected by the developing situation in many segments. Many countries are a good example of approval. Corruption can lead to uncontrolled contamination of water sources. It is necessary to have legal frameworks and effective policies that must support for water management goals. But, despite acceptable policies and legislation, the development of water resources, however, requires adequate financing of infrastructure and appropriate human skills (UNESCO 2009). Strong institutions that protect justice to save the population from any disease originating from polluted water.

My topic responds to environmental problems in Georgia. Strategic document "III National Program of Georgian environmental protection" (2017-2021 years) – elaborated by the Ministry of Environment Protection and Agriculture of Georgia, points to the topicality of the problem of environmental pollution in Georgia (MEPAG 2019).

Given work is focused on the set-up of a new technological approach on the bioaccumulation and removal of heavy metals from wastewater, based on the effective sorption properties of Algae *Spirulina Platensis* and chelating agents, in order to improve the phytoremediation technology.

3. Materials and Methods

3.1. Materials

3.1.1. Biomass of Spirulina *Arthrospira platensis*

In the experiments were used the biomass *Spirulina platensis* obtained via cultivation in standard Zarrouk's medium (pH – 8.7; content in g/L: $NaHCO_3$ – 16.8, K_2HPO_4 - 0.5, $NaNO_3$ – 2.5, K_2SO_4 – 1.0, NaCl – 1.0, $MgSO_4 \cdot 7H_2O$ – 0.2, $CaCl_2 \cdot 2H_2O$ – 0.04, $FeSO_4 \cdot 7H_2O$ – 0.01, EDTA – 0.08; and microelements kit A5 – 1 mL). The incubation was carried out with permanent air barbotage (rate of air flow 2 L/min), at temperature 25°C, and under following illumination conditions: a photoperiod of lighting 16L/8D (16 hours of light: 8 hours of dark), a total photosynthetic photon flux density (PPFD) of ≈15 μmol. m^{-2}. s^{-1}.

3.1.2. Chemicals

$NaHCO_3$, $K_2HPO_4 \cdot 3H_2O$, $NaNO_3$, K_2SO_4, NaCl, $MgSO_4 \cdot 7H_2O$, $CaCl_2 \cdot 6H_2O$, Fe, EDTA, $FeSO_4 \cdot 7H_2O$, H_3BO_3, $MnCl_2 \cdot 4H_2O$, $ZnSO_4 \cdot 7H_2O$, $CuSO_4 \cdot 5H_2O$, MoO_3, NH_4VO_3, $K_2Cr_2(SO_4)_3 \cdot 24H_2O$, $NiSO_4 \cdot 7H_2O$, $Na_2WO_4 \cdot 2H_2O$, $Ti_2(SO_4)_3$, $Co(NO_3)_2 \cdot 6H_2O$, KOH, $Pb(CH_3COO)_2$, HNO_3, HCl were of analytical grade and were purchased from Shota Rustaveli National Science Foundation (SRNSF) and Volkswagen Foundation, the Doctoral Program "Sustainable and Agricultural Food Systems" (SAFS).

3.1.3. Reservoirs, flasks, pipettes, thermometer with thermostats, oxygen and daylight sets

Reservoirs (volumes: 20L. 15L and 5L), flasks, pipettes, thermometer with thermostats, oxygen and daylight sets were used to provide the constant temperature, oxygen, and lighting mode.

3.2. Equipment

3.2.1. pH meter

Model: S-903 Portable, display-3 1/2-digit LCD. The pH meter was used to determine the acidity of the Spirulina in the incubation area.

3.2.2. Stirrer

Model: Zenith Lab. Ceramic magnetic stirrer (MHS-A) Product name: Ceramic magnetic stirrer; Model: MHS-A; Speed range: start up; ~1600 rpm stepless speed adjustment; Speed display: Yes; Heating power: 600W
Stirring power: 60W; Temperature range:-50~320℃; Temperature precision: ±0.1℃
Timer: 0-9999min; Working plate size(mm): 180x180mm; Voltage: 220V 50Hz/ 110v 60Hz; Rotating speed: 40-1600 rpm; Characteristic: Microprocessor control, Digital display speed, time, temperature.

3.2.3. Centrifuge

Model: HIGH SPEED CENTRIFUGE (HC-16A); Strong metal case; Brushless motor, LED display speed RCF and time; Available different rotors; Over speed and imbalance alarm; Rotor identification, electromagnetic door locks; Touch panel control, the error code display; Stainless steel chamber; Suitable for experiments and researches of biology, pharmacology and agriculture; For researches on genes and proteinnucleic acid; LCD display optional.

3.2.4. Biosurfactants

Rhamnolipids-RL1 and RL2-Microbial synthesis of the rhamnolipid biosurfactants was conducted using the *Pseudomonas* sp. PS-17 strain from collection of Department of Physical Chemistry of Fossil Fuels of InPOCCC, National Academy of Sciences of Ukraine.

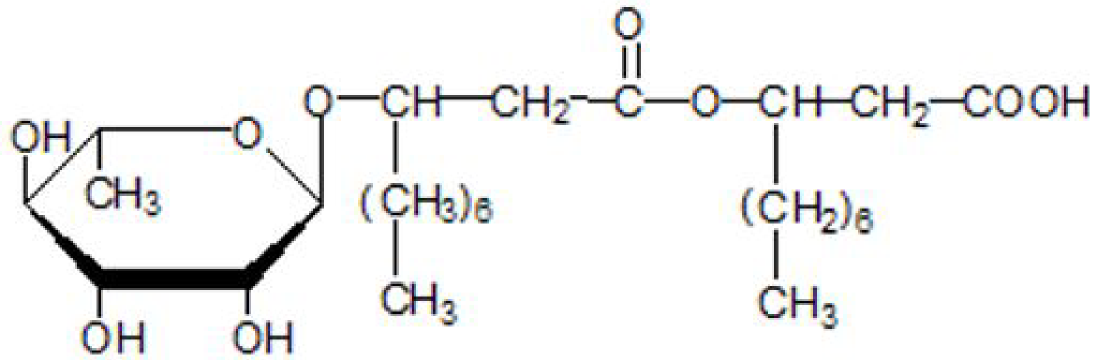

Fig.1a. Rhamnolipid1

RL1- α-L-Rhamnopyranosyl-β-hydroxydecanoyl-β-hydroxydecanoate

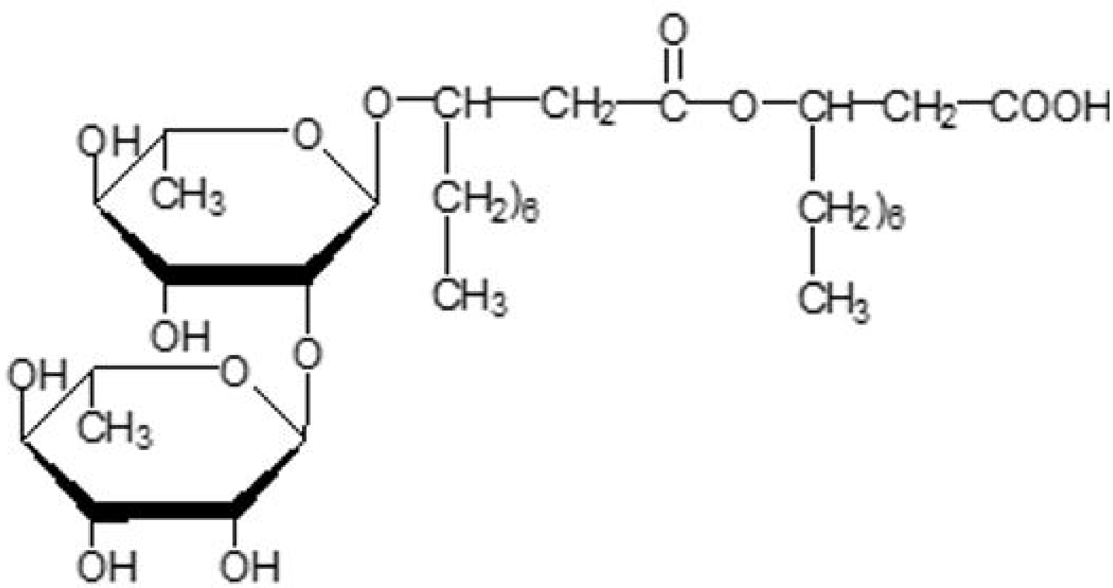

Fig. 1b. Rhamnolipid2

RL2- α-L-Rhamnopyranosyl- α-L-rhamnopyranosyl -β-hydroxydecanoyl-β-hydroxydecanoate

Trehalose lipid- TRL-Biosynthesis of the trehalose lipid biosurfactants was conducted using the *Rhodococcus erythropolis* AU-1 strain from the Ukrainian collection of microorganisms of D. Zabolotny Institute of Microbiology and Virology, National Academy of Sciences of Ukraine.

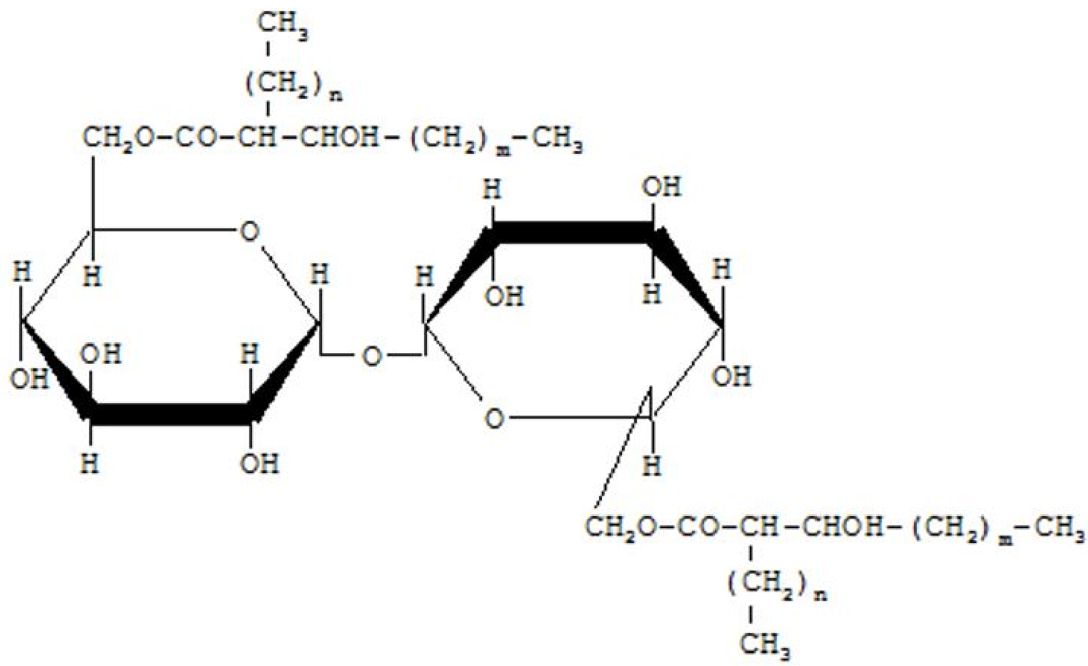

Fig.1c. Trehalose Lipid

TRL- two glucose molecules inter linked by α (1—1) glycoside bond

3.2.5. UV-visible Spectrophotometer

Model: VISIBLE SPECTROPHOTOMETER UV722 Large LCD screen (128*64 Dots) with saving standard curve function
Imported & Environmental Deuterium lamp, preventing from Ozone inhalation
Adapted high-class grating with wholly hermetic light path design, to ensure the instrument has a super low stray light
Real-time monitoring the lifetime of Deuterium lamp and Tungsten lamp with advanced system
Pre-aligned design ensures the user can change lamps conveniently
Wavelength calibration, wavelength setting, change lamp source and dark current calibration automatically with Si02 coating optical mirror, reducing the pollution from outside
Optional PC Software to expand the applications to Quantitative, Multi-Wavelength and Kinetics
Widely used for organic chemistry, inorganic chemistry, life sciences, food, medicine, health, agriculture, geology, metallurgy and environment fields.

Spectrophotometer was used to determine concentrations of Biomass and chlorophyll in the incubation area, and to measured heavy metals number in the incubation area (solution) of Spirulina.

3.2.6. Atomic Adsorption Spectrophotometer

An Atomic Absorption Spectrophotometer – Perkin Elmer A Analyst 200
Complete control of the AAnalyst™ 200 through an innovative touch-screen interface. Flexible software for lamp setup, flame control, parameter selection and sample analysis. Display- Full-color 10.4" LCD touch screen. VGA resolution (640 x 480 pixels). Coated for maximum durability and lifetime. The sample-analysis page is used for manual or automated analysis. All results are displayed on the screen. Sample-identification numbers can be added. The calibration curve can be displayed and printed with correlation coefficient, slope and intercept. Peaks can be displayed and printed when using the MHS-15 accessory. Automated analysis can be performed with the AS-90plus or AS-93plus autosampler. The MHS-15 can be used for the determination of hydride-forming elements and mercury. Echelle monochromator. Focal length: 300 mm. Grating: 36 x 185 mm area, 79 lines/mm, blaze angle 76°. Fused quartz prism: 95 x 40 mm, 60°. Wavelength range: 189-900 nm.
Spectral bandpass: 0.15 nm at 200 nm. The photometer optics are covered to protect against dust and corrosive vapors. For maximum protection, the optical system can be purged with an inert gas.

An Atomic Absorption Spectrophotometer was used to measured concentrations of heavy metals (Cu^{2+} and Pb^{2+} ions) in the Spirulina biomass.

3.3. Methods

The laboratory of biological oxidation of the Institute of Biochemistry and Biotechnology of the Agrarian University has carried out a fundamental study, the main purpose of which is to assess the resistance of Spirulina to detergents of various chemical nature and its detoxifying ability. The main essence of the scientific and technical research approach is to effectively manage the process of assimilation of ecotoxicants by Spirulina.

The presented research was carried out from April 2019 to October 2021. Experiments provided in the glass tanks (vol. 40 l). The optimal conditions for cultivation of Spirulina in Cu^{2+}-containing medium were chosen. As a result, a solution of $CuSO_4$ in 0.1 M phosphate buffer (pH—6.0) was selected. The other optimal conditions are as follows: temperature of cultivation— 25 °C; daylight illumination; duration of incubation— 72 h.

3.3.1. Determining optimal conditions for Spirulina biomass

At the initial stage I developed the literature and got acquainted with the conditions of Spirulina cultivation for high productivity (Zhang et al. 2015). Given that nutrition involves determining the optimal mineral composition and Sodium bicarbonate plays an important role among them - we observed different variations in the amount of soda in the incubation area, resulting in the selection of the ultimate optimal dose.

We also conducted an experiment and grow Spirulina in saline. For this was prepared a physiological solution of 0.9% (5 L of water and 45 g of NaCl (sodium chloride)).

Was chosen the optimal temperature; Set the lighting mode; Determine the optimal pH value, which performed with pH meter-S-903 Portable Model, display-3 1/2-digit LCD,

To observe the biomass performance of Spirulina, the biomass was permanently tested using a **UV-visible spectrophotometry** as follows: Because the samples taken from the Spirulina incubation zone were non-homogeneous suspension, we performed the sampling using a **magnetic stirrer (MHS-A)** to make sure that all samples were taken under the same conditions and the difference between them was minimized. For this experiment was taken a 20 ml sample from the incubation solution and was stirring for 2 minutes, from which we took the final sample for analysis as 3 ml, which was measured on the **UV-visible spectrophotometry** at 750 nm (Butterwick 1982).

Balance of the AS R1 PLUS series

A standard analytical weighing device was used to weigh each ingredient in the experiments.

3.3.2. The Selection of the Required Concentration

For investigations, the concentrations 1, 10, and 100 mg/L of Cu^{2+} (as copper (II) sulfate) were chosen. For study of the dynamic of Cu^{2+} content in incubation medium during Spirulina cultivation, the following method was elaborated: Cu^{2+} content was determined according to cuprizone method (Marczenko and Balcerzak 2000) spectrophotometrically at 600 nm. After1,3,6,24, and72hfrombeginningofincubation,4ml sample of incubation medium is taken and is added to solution of Cuprizone (bis-cyclohexanoneoxyldihydrazone) and ammonia (pH of solution is 8– 9.5). For stimulation of glutathione S-transferase activity in the incubation area of Spirulina with Cu^{2+} was added glutathione. After 2 h of incubation, whole sample was centrifuged at 1000g during 20min. In obtained supernatant, Cu^{2+} content is determined spectrophotometrically.

3.3.3. The influence of Cu^{2+} and Pb^{2+} ions on the formation of biomass and chlorophyll of Spirulina

Experiments were conducted for selection of the optimal concentration of heavy metals in the laboratory, which will not significantly affect maintaining the physiological parameters of Spirulina.

The biomass of Spirulina *platensis* has been got via cultivation in standard Zarrouk's medium (pH–8.7; content in g/L: $NaHCO_3$ – 16.8, K_2HPO_4 – 0.5, $NaNO_3$ – 2.5, K_2SO_4 – 1.0, NaCl – 1.0, $MgSO_4{\cdot}7H_2O$ – 0.2, $CaCl_2{\cdot}2H_2O$ – 0.04, $FeSO_4{\cdot}7H_2O$ – 0.01, EDTA – 0.08; and microelements kit A5 – 1 mL). The incubation was carried out with permanent air barbotage (rate of airflow 2 L/min), at temperature 25°C, and under following illumination conditions: a photoperiod of lighting 16L/8D (16 hours of light: 8 hours of dark), a total photosynthetic photon flux density (PPFD) of » 15 μmol m^{-2} s^{-1}.

From many experiments on the selection of the maximum concentration of metals, at which there are no significant changes in the physiologically important parameters of Spirulina – the formation of biomass and the production of chlorophyll, a concentration of 100 ppm of metal ions was chosen. The presented studies were carried out at concentrations of 100 ppm for both metals - copper and lead. Experiments were provided in the glass tanks (vol. 20 L). For cultivation of Spirulina in Cu^{2+}-containing medium were chosen $CuSO_4$ and as Pb^{2+}-containing medium was selected Pb $(NO_3)_2$. To measure the effect on biomass at the initial moment, the biomass of *Arthrospira platensis* was 10 g/L and the other optimal conditions were the temperature of cultivation – 25°C; Daylight illumination; duration of incubation – 120 h. The biomass of *Arthrospira platensis* in incubation medium has been measured spectrophotometrically at 750 nm (Butterwick et al. 1982).

As for the influence on form chlorophyll when water is contaminated with heavy metals, in the control sample (without contamination) chlorophyll was 8 mg/g of fresh Spirulina biomass.
For measurement of fresh biomass productivity and chlorophyll formation by Spirulina, the following method was elaborated: the incubation medium was centrifuged at 1000 g for 20 min, and the obtained pellet was weighted. The got fresh biomass was treated with acetone and contain chlorophyll was determined spectrophotometrically at 652 nm according to the standard method (Arnon 1949). The tests on chlorophyll and biomass were carried out at 50ppm and 100 ppm for both metals (Copper and Lead).

To provide rough check for the determination of total chlorophyll the light absorption date for chlorophyll were plotted and the curves were found to intersect at λ = 652mμ. By extrapolation, the value of specific absorption coefficient for this wavelength was found to be 34.5. Another equation was therefore set up for total chlorophyll:

D_{652}=34.5C+34.5C_b=34.5(C_a+C_b)

$C=(C_a+C_b) = \frac{D652}{34.5}$

Or expressed as mgs. Per liter:

$$C = \frac{D652X1000}{34.5}$$

3.3.4. The Influence of Glutathione on Chelating of Cu^{2+} Ions

The glutathione S-transferases (GSTs) are an abundant family of dimeric proteins that have the capacity to conjugate glutathione (GSH) with a variety of compounds containing electrophilic centers (Croom 2012). Glutathione-S-Transferase (chemically it is sulfur-containing three peptides), participates in metal conjugation for plants. By the disulfide bridge, they bind metals and provide penetration in the cell.

To study the influence of Cu^{2+} on cell ultrastructure, the biomass of Spirulina was fixed in 2.5% glutaraldehyde and then in 1% OsO_4. After dehydration in ethanol of increasing concentrations, the samples were embedded in Epon–Araldite resin (1.5:1.0) and poured into gelatin capsules (Buadze et al. 1998). Spirulina biomass ranged from 3.0 to 4.5 g/L. Incubation was carried out. in a glass aquarium with sizes 60×21×40 (in cm, length × width × height), with constant air barbotage (rate of air flow 2 l/min), at a temperature of 25 °C, under the following lighting conditions: 24 l/0 D, PPFD ≈ 15 μmol m–2 s–1. In the control variant, instead of the tested contaminant solution, we added 20 l of water.

3.3.5. The Study with Electron Microscope

Thin serial sections (50–60 nm) were made using an LKB III ultra-microtome, stained with 2% uranyl acetate, and examined in an electron microscope Tesla BS 500 (Czech Republic) (specifics, transmission; resolution, 0.8 nm).

3.3.6. The uptake of different concentrations of Cu^{2+} and Pb^{2+} from cultivation area by Spirulina has been studied.

The incubation was carried out in a glass container with sizes 60 x 21 x 40 (in cm, length x width x height), with permanent air barbotage (rate of air flow 2 L/min), at temperature 25°C, under following illumination conditions: 24L/0D, PPFD ≈15 µmol. m^{-2}. s^{-1}. In the control variant instead of tested contaminant solution was added 20 L of water.

According this experiments concentration of pollutants in solution after 14 days' incubation time was following: For Cu^{2+} - 22.97ppm or 19.55ppm and For Pb^{2+} - 19.75ppm or 15.22ppm. Initial concentration for these solutions was 100 ppm. The biomass of Spirulina varied from 3.0 to 4.5 g/L (see Table 1 and Table 2).

Number of model experiment	Initial content of Spirulina in polluted water g / L	Content of Cu^{2+} ions in polluted water (ppm)	
		Initial (before incubation)	Final (after incubation)
Cu-1	3.0	10	7.72± 0.95
Cu-2	3.5	10	7.53 ± 0.08
Cu-3	4.0	100	22.97 ± 0.06
Cu-4	4.5	100	20.75 ± 1.16

Table 1. Results of model experiments for cleaning water polluted with Cu^{2+} ions by using Spirulina (incubation period 14 days)

Numberof model experiment	Initial content of Spirulina in polluted water g / L	Content of Pb^{2+} ions in polluted water (ppm)	
		Initial (before incubation)	Final (after incubation)
Pb-1	3.0	10	6.11 ± 0.71
Pb-2	3.5	10	5.23 ± 0.23
Pb-3	4.0	100	19.75 ± 0.08
Pb-4	4.5	100	15.22 ± 0.98

Table 2. Results of model experiments for cleaning water polluted with Pb $^{2+}$ ions by using Spirulina (incubation period 14 days)

Each experiment was repeated 3 times. Data presented represent the mean of triplicates ± standard deviation. The statistics were subjected to one-way analysis of variance. Calculations of the obtained results were performed in Microsoft Excel.

3.3.7. Study of the dynamics of Cu^{2+} and Pb^{2+} ions uptake by Spirulina cells Atomic adsorption spectrophotometry)

Cu^{2+} and Pb^{2+} content is determined by the method of atomic absorption (flame emission) analysis (GOST 30178-1996). Conditions for analysis are following: Instrument – Perkin Elmer A Analyst 200; wavelength – 324.8 nm and 283.3 nm for Cu and Pb, respectively; Fuel gas – C_2H_2-Air (Table 3).

Chemical	Wavelength, nm	Determination, µg	Fuel gas	Oxidizing
Cu	324.8	0,2	C_2H_2 - Air	No
Pb	283.3	0,2	C_2H_2 - Air	No

Table 3. Conditions for analysis are following: Instrument – Perkin Elmer A Analyst 200

Sampling and analysis of air-dried sludge material was performed by standard method, using atomic-absorption spectral analysis. 1^{st} and 2^{nd} samples were taken after 4 and 8 hours on the first day and others once a day on subsequent days.

The disintegration of the samples was carried out according to GOST 30178-1996 by the dry sieving method. Air-dried samples are weighed and placed on porcelain jars. After baking the samples in the muffle oven at 550^0C for 2-3 hours, add a few drops of concentrated nitric acid to the dry residue and place them in the muffle again for 20-30 minutes. The procedure is repeated until the dry residue turns yellowish or reddish on entry and no more black charcoal particles appear. Then 5 ml of hydrochloric acid is added to the cooled sample in a ratio of 1: 1, carefully placed on an open stove, carefully stirred with a glass beaker, the solution is transferred to a 25 ml flask and filled with distilled water. In the same way, a zero solution is prepared using a blank sum. The content of Cu^{2+} and Pb^{2+} ions in the obtained solutions is determined by the atomic absorption method.

Preparation of standard caliber series: The caliber series is made from tested standard solutions with quality certification documentation. Perkin Elmer certified standard solutions are used in Gamma Lab.

Calibrated series with the following concentrations of study elements were prepared for analysis: 0.2; 0.5; 1.0; 2.0; 4.0; 6.0; 8.0; 10.0 µg / ml. This device automatically bears the caliber curve and calculates the concentration of the solution. The concentration of elements in the solutions of the study samples is given in µg / ml.

To exclude the number of heavy metals that can pass into sediments, a test was carried out directly on the biomass of *Spirulina platensis* to measure Cu^{2+} and Pb^{2+} content by atomic absorption methods. Tests were carried out on artificially contaminated water with a concentration of 100 ppm of Pb^{2+} and 100 ppm Cu^{2+} respectively. Samples were taken every 4 hours on the first day and once a day for the next four days. A control sample was taken before heavy metal contamination of the water. Spirulina biomass ranged from 4.0 to 4.5 g/L. Disintegrate samples were carried out according to GOST 30178-1996 by ash method (Caroli 2006; Interstandard 2013). Air-dried samples were weighed and placed on porcelain jars. After firing the samples in a muffle furnace at 550° C for 2-3 hours, a few drops of concentrated nitric acid were added to the dry residue and again placed in a muffle for 20-30 minutes. The procedure is repeated until the dry residue turns yellowish and the particles of black coal cease to appear. Then, 5 ml of hydrochloric acid was added to the cooled sample in a 1/1 ratio. The solution was transferred to a 25 ml flask and filled with distilled water. In the same way, a null solution was prepared using an empty amount. The samples were analyzed using an Atomic Absorption Spectrophotometer – Perkin Elmer A Analyst 200.

3.3.8. Addition of EDTA to determine the effect of the ability of Spirulina on the absorption of heavy metals

Because of the chelating properties of EDTA, it has been useful to figure out how to increase the absorption capacity of *Arthrospira platensis* about heavy metals. Thus, laboratory analyzes were performed on Spirulina plus EDTA to compare the number of heavy metals penetrate the Spirulina mass over the next 120 hours.

Spirulina biomass ranged from 10 mg L^{-1}. The incubation was carried out in a 50 × 20 × 25 glass aquarium (in cm, length × width × height) with constant air barbotage (air flow rate 2 L min^{-1}), temperature 25°C, with the following lighting conditions: 24 L/0 D, PPFD ≈ 15 $\mu mol \cdot m^{-2} \cdot s^{-1}$. These experiments were carried out on both heavy metals, copper and lead to be investigated. A sample of *Arthrospira platensis* was taken from the incubation area for test in 1-liter flasks. There were added salts $CuSO_4$ and $Pb(NO_3)_2$ respectively, for artificial pollution with copper and lead (100 ppm). Two control samples were taken, to check the metal content in them. Control a0

(*Arthrospira platensis),* and Sample b0 (*Spirulina platensis* + EDTA). In the other samples, the chelating agent was added twice than copper and lead accordingly. Sampling was carried out for 4 hours, 1 time during the first day and the following days, 1 time per day, including the fifth day. 20 ml. Sample was centrifuged for 5 min at 1000 g. to get 2 g of Spirulina biomass. Contain copper and lead were determined by the atomic absorption method in the got mass of Spirulina.

3.3.9. The influence of biosurfactants on the absorption of heavy metals (Cu^{2+} and Pb^{2+}) by Spirulina

Microbial synthesis of the Rhamnolipid biosurfactants was conducted using the *Pseudomonas* sp. PS-17 strain (from the collection of Department of Physical Chemistry of Fossil Fuels of InPOCCC, National Academy of Sciences of Ukraine). The strain synthesizes homologous extracellular Rhamnolipids and extracellular biopolymer, which form a surface-active complex with Rhamnolipids (Semeniuk et al. 2020).

The Rhamnolipids, RL1, and RL2, contain one and two Rhamnose residues respectively, and two residues of 1-β-hydroxidecanoic acids. Containing of RLs and TRL were determined spectrophotometrically (UVmini–1240, Shimadzu, Japan) using the orcinol method. The Rhamnolipids were isolated by extraction with Folch mixture (chloroform-methanol 2:1) which was further separated and analyzed using thin-layer chromatography (Lubenets et al. 2013).

For finding out the effect of biosurfactants, two different Rhamnolipids were used: Rhamnolipid1 (mono-Rhamnolipid) and Rhamnolipid2 (di-Rhamnolipid), also Trehalose lipid. Spirulina biomass was sampled in the one-liter flask to analyze each biosurfactant and heavy metals. For artificial pollution with copper and lead, lead nitrate and copper sulfate salts were used, respectively. Analysis were carried out for three kinds of biosurfactants in different flasks. The number of biosurfactants was added to 0.01% of the total solution.

The combination of analytical samples was distributed: Spirulina+copper+RL1; Spirulina +copper+RL2; Spirulina+copper+TRL; Spirulina+lead+RL1; Spirulina+lead+RL2; Spiruluna+lead+TRL.

Analytical samples were taken 4 hours, 8 hours, 24 hours, 48 hours, 72 hours, 96 hours, and 120 hours after application of the biosurfactants. The analyses were performed by the atomic absorption method. The analysis was performed on a Perkin Elmer atomic-absorption spectrometer A Analyst 200. The determination conditions are given in the table.

The computation of results was carried out in Microsoft Excel (Version 2019). The statistics were subjected to a one-way analysis of variance. The data presented represents the mean of triplicates ± standard deviation. Statistical analysis was done by ANOVA.

Expressing results

The results obtained are expressed in mg / kg and are calculated using the formula:

$$C_{el} = \frac{A*V}{g}$$

where:

A - Concentration of the investigated element in solution, μg / ml

V- is the volume of the test solution, ml

g - is the mass of the test sample, g.

Results of different concentration Cu^{2+} and Pb^{2+} ions on the formation of biomass and chlorophyll of Spirulina

4. Result and Discussion

4.1. Optimal Conditions of Spirulina Biomass

Experimental stages carried out by me during 4 years can be divided into 4 levels.

In the first stage, we aimed to select an incubation environment that would give us the highest possible biomass and help Spirulina to multiply rapidly and produce strong biomass. Which would be an unmistakable precondition for the more efficient implementation of our task - the absorption of more heavy metal from polluted water.

The model experiments for development of the phytoremediation technology based on the application of *Arthrospira* for cleaning chemically polluted waters were carried out. In the model experiments, the components of the initial incubation medium of biomass of Spirulina were changed until we got the maximal productivity of the *Arthrospira platensis.*

Given that nutrition involves determining the most favorable mineral constitution and Sodium bicarbonate has an important part among them - we observed different variations in the quantity of soda in the incubation area, consequence in the selection of the last optimal amount was chosen 16.8 g (Tabagari et al. 2019).

At the initial stage of this work, the main task was to obtain a large biomass of Spirulina by varying the content of the nutrient medium and the duration of these experiments was 120 days. But when switched to studying the ability of Spirulina to assimilate copper cations, it turned out that the absorption generally lasted 72 hours (Tabagari et al. 2020).

Since Spirulina reproduces well in salt water (Kumar et al. 2020; Palaniswamy and Veluchamy 2017), we decided to conduct experiments which medium is more productive: Spirulina: Zarrouk's or saline area. An experiment was performed and we compared chlorophyll production and biomass grown in two different mediums.

Comparison of Zarrouk's and Physiological solution in Biomass Productivity and Chlorophyll Production of Spirulina shown in diagram Fig.1a and Fig.1b.

As shown in **Figure 2a**, after 14 days the Spirulina biomass in the Zarrouk's medium increased 2 times better than in the area of physiological solution, and regarding the chlorophyll content it was 20% lower in the salted water then Zarrouk's area **Figure 2b.**

In the Zarrouk's medium the pH was 8.7, and in the physiological solution-7.2 for 14 days.

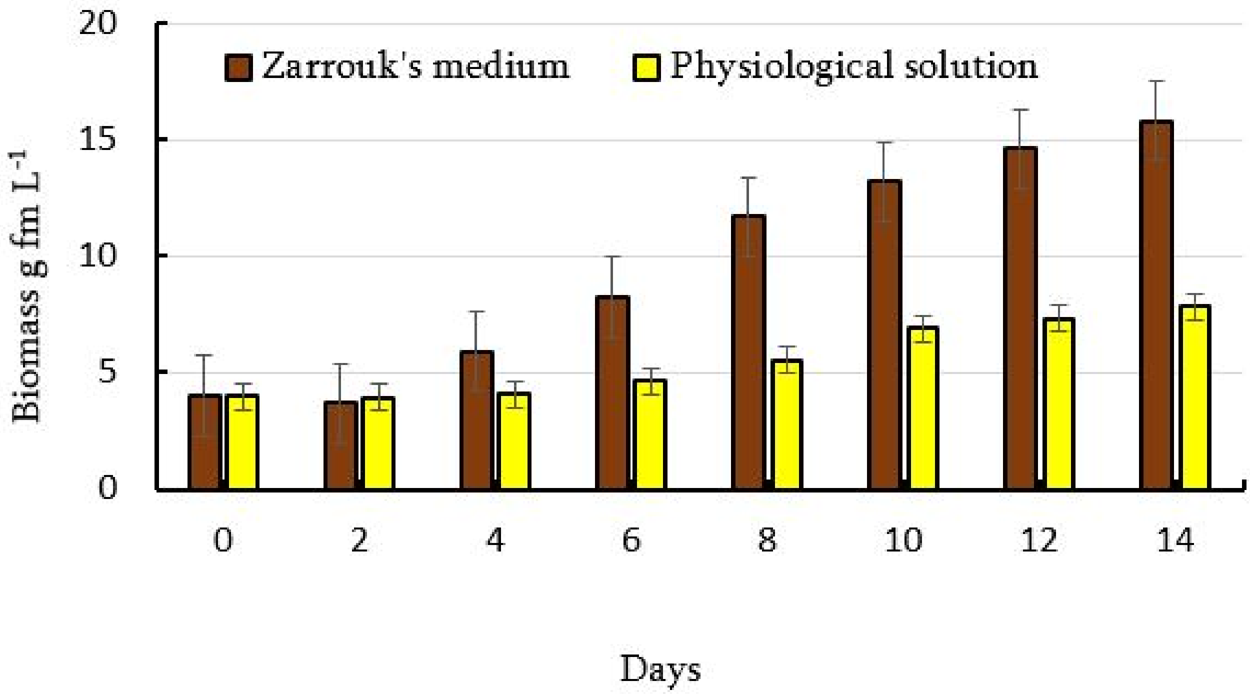

Fig. 2a. Spirulina biomass in the area of physiological solution -
Spirulina biomass in the Zarrouk's medium increased 2 times better than in the area of physiological solution

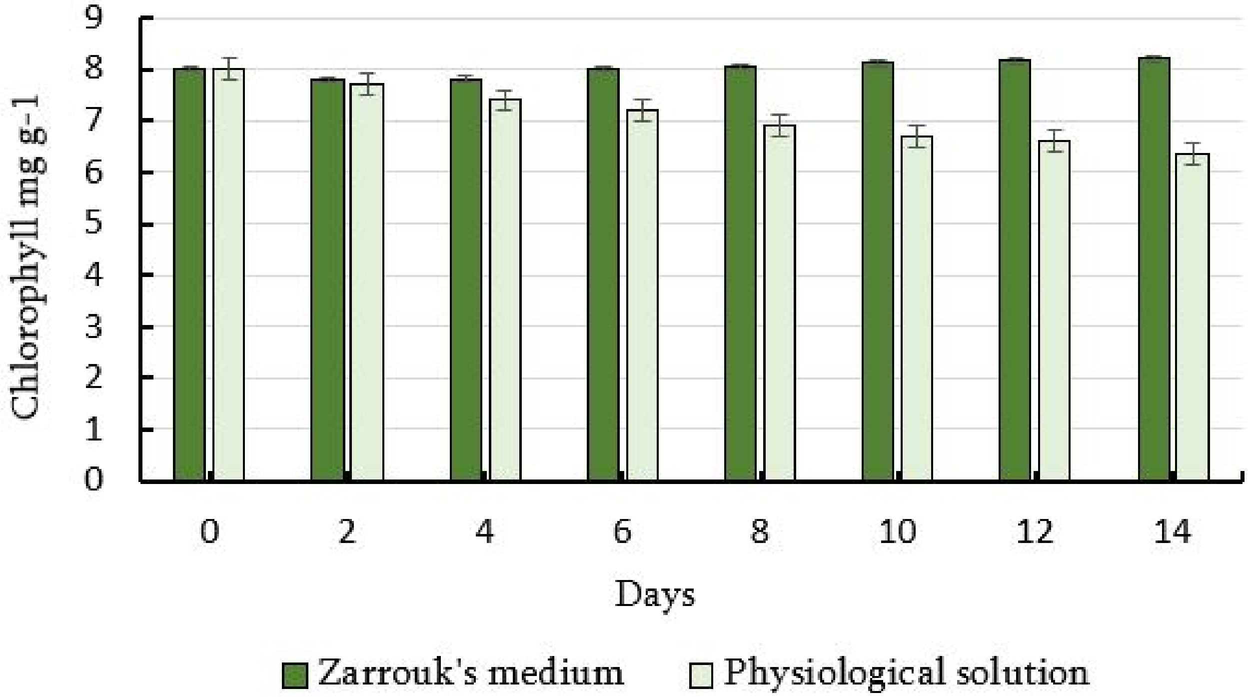

Fig.2b. The chlorophyll content in the salted water –

The chlorophyll content is 20% lower in the salted water then Zarrouk's area

Although information and works are often found for wastewater treatment of heavy metals and microorganisms, where *Arthrospira platensis* is a key bio-tool, the presented work contains some novelty. Removing such persistent copper and lead ions from water using the ecological potential of *Arthrospira* is the first attempt and has no direct analogy.

4.2. Doses Selected for Experiments

At the initial stage of any study, it is important to determine the dose of the toxicant at which the physiological parameters of the subject will be maintained.

The second and most important step was to select the concentrations required in which the incubation area would be artificially contaminated with heavy metals to maximize the physiological parameters of Spirulina (biomass generation and chlorophyll accumulation) with the best expressed heavy metals. Initially, experiments were performed on copper-contaminated water. Different concentrations of 1, 10, and 100 ppm were selected for comparison at the initial stage.

The goal was to select the metal concentration, which does not cause noticeable shifts in the physiology of cyanobacteria and slightly affects the homeostasis of this biosystem. The figures show the effects of copper on Spirulina biomass production and chlorophyll accumulation. The figures show the effects of copper on Spirulina biomass production and chlorophyll accumulation. The highest concentration of Cu^{2+}-ions (100 ppm), which causes the inhibition of biomass accumulation by 15% (Fig. 3a) and the decrease of chlorophyll content by 30% (Fig. 3b), has been chosen for the investigations.

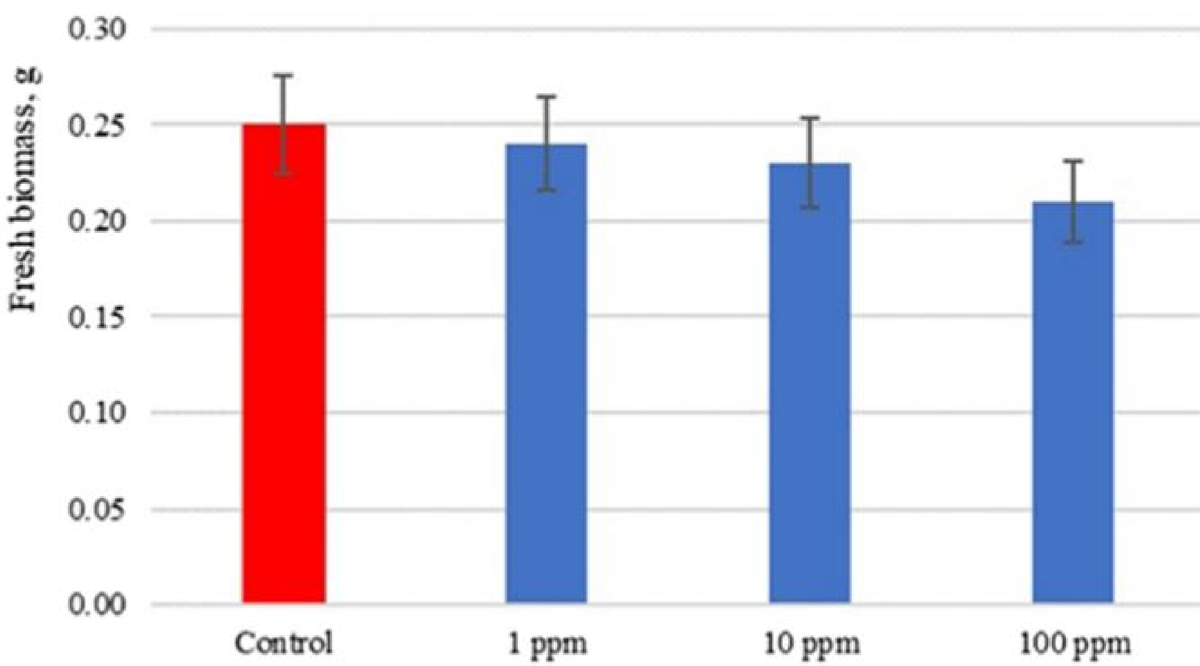

Fig. 3a. The influence of different concentrations of Cu^{2+} ions on biomass
The data on (a) shows influence of Cu^{2+} ions on fresh biomass (Control 0.25 g, 1 ppm 0.24 g, 10 ppm 0.23 g,100ppm 0.21g).

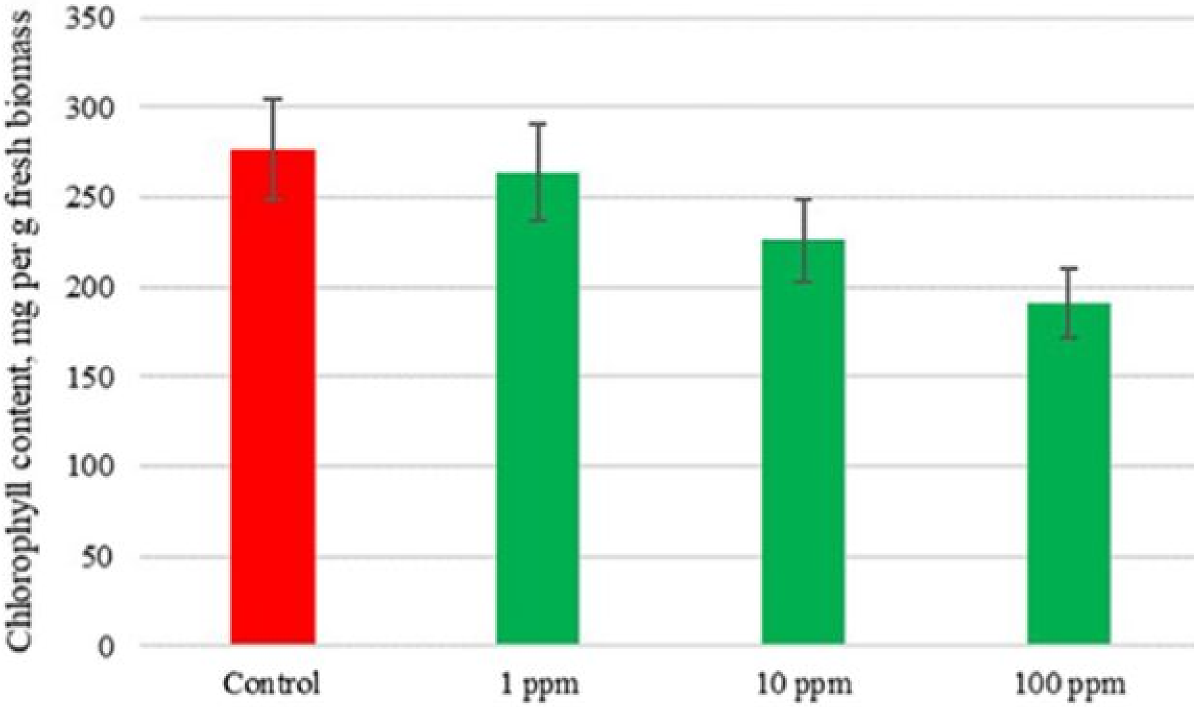

Fig. 3b. Chlorophyll accumulation by Spirulina after120-h incubation

The data on shows in fluence of Cu^{2+} ions on chlorophyll accumulation by Spirulina (Control 275 mg, 1 ppm 260 mg, 10 ppm 225 mg, 100 ppm 190 mg)

Based on observations of the experiments, since the sample with 1 ppm did not have significant informative results, we continued the study of samples with 10 ppm and 100 ppm (**Fig. 4**). As it follows from Figure 4, the sample with 10 ppm uptakes 21% and sample with 100 ppm uptakes 70% of copper from the contaminated water (Tabagari et al. 2020).

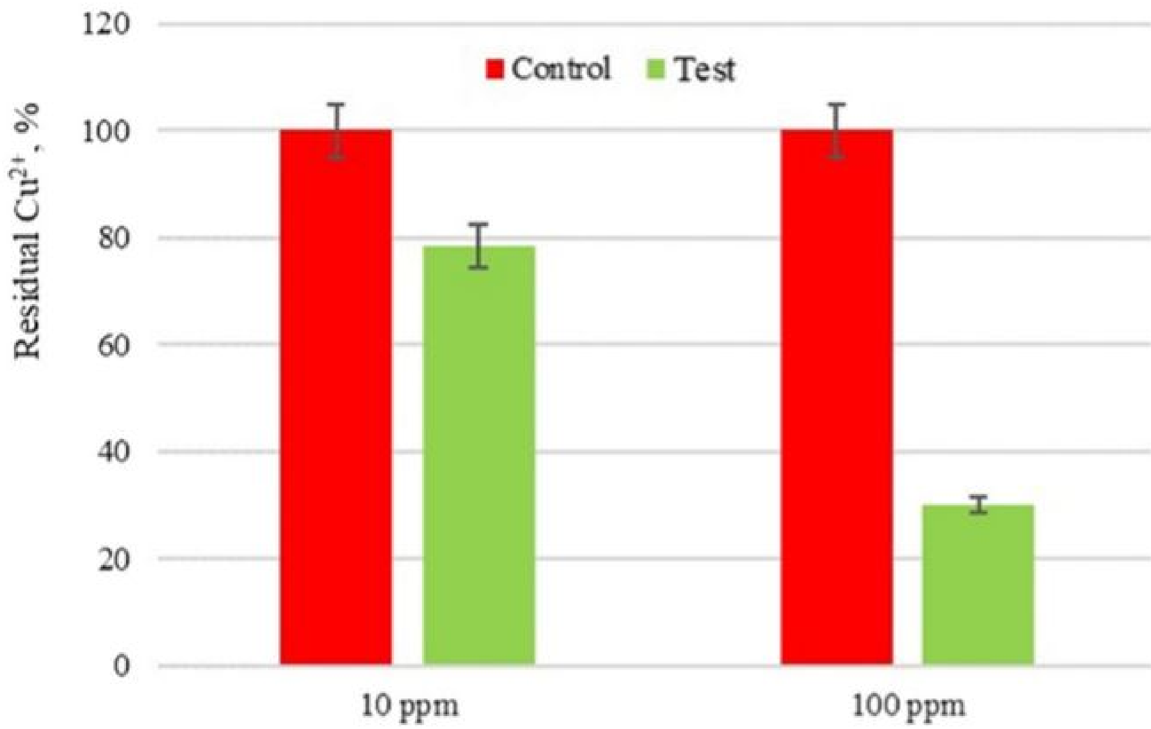

Fig. 4. The variation of Cu concentration in aqueous solution under the influence of contamination level and algal activity.

The data are presented residual Cu^{2+} ions in 10 ppm (Control 100%, Test 79%) and 100 ppm (Control 100%, Test 30%)

4.3. Results of influence of Glutathione (GSH) on chelating of Cu^{2+} Ions

For investigation of detoxification processes of Cu^{2+}: Ions in Spirulina need to be established what enzymes participating in Cu^{2+}-ions utilization. For this aim it is necessary to study what enzymes of Spirulina undergo induction after growing on toxicant-containing area. According to literature (Buadze 1998) glutathione S-transferase an inducer of chelation of heavy metal cations can be used as an indicator.

At the first stage of research, the methods for determination of activities and induction degree of above-mentioned enzyme has been elaborated. The method is as follows: Spirulina were growing on Cu^{2+}-ions – containing area, the activity of enzyme was measured after 1, 3 and 5 days in suspension. For establishment of induction degree, the activities of enzymes have been compared with the control in which Spirulina was growing without toxicant.

The stimulation of glutathione S-transferase at incubation of Spirulina with Cu^{2+} has been studied. The obtained results show that glutathione significantly reduces (approximately 6-fold) the content of Cu^{2+} in the incubation area that indicates the possible participating of glutathione S-transferase in Cu^{2+} utilization process by Spirulina cells.

The mixture containing Cu^{2+}-ions with enzyme preparation of Spirulina and different concentrations of GSH was incubated during 1hour, at temperature 25°C. After incubation, the whole sample was centrifuged at 1000 g during 20 min. In obtained supernatant, Cu^{2+} content is determined spectrophotometrically. The obtained results are presented in Figure 3.

Concentrations of components in incubation mixtures (5 ml): physiological solution (0.9% of NaCl), enzyme preparation from Spirulina - 0.6 mg protein per ml, Cu^{2+}-ions – 400 ppm, GSH – 0.06, 0.12 and 0.24 M. Conditions of incubation: 25°C, 1 hour.
Test variants, presented on **Figure 5.**

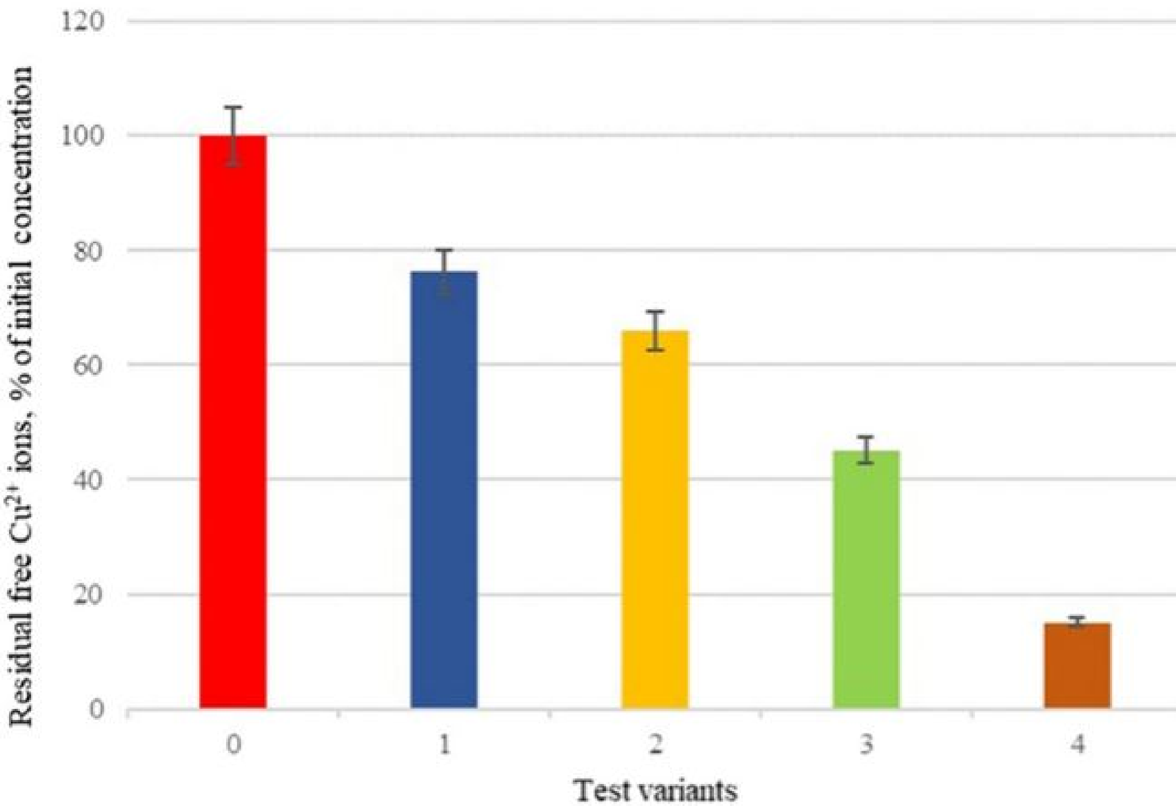

Fig. 5. The influence of different concentrations of glutathione (GSH) on chelating of Cu^{2+} ions by enzyme preparation, isolated from the biomass of Spirulina.

Comparative analysis shows influence of glutathione on chelating of Cu^{2+} ions by enzyme preparation, 4 test variants: Control 100%; 1 variant 78%; 2 variant 63%; 3 variant 42%; 4 variant 18%

0 – Blank variant (Cu^{2+} ions incubated without enzyme preparation)

1 – Cu^{2+} ions incubated with enzyme preparation (Control variant)

2 – Cu^{2+} ions incubated with enzyme preparation and 0.06 M of GSH

3 – Cu^{2+} ions incubated with enzyme preparation and 0.12 M of GSH

4 – Cu^{2+} ions incubated with enzyme preparation and 0.24 M of GSH

The obtained results show that high concentrations of glutathione significantly reduce the content of free Cu^{2+} in the incubation area that clearly indicates participation of glutathione S-transferase in Cu^{2+} salvaging process by Spirulina cells (Tabagari et al. 2020).

4.4. Electron Micrographs of Spirulina

The influence of Cu^{2+}-ions on intracellular structure of Spirulina cells have been studied by electron microscopic method. An electronic cell study revealed the process of copper absorption by green Algae in dynamics. The micrograph taken on the first day clearly shows location of heavy metal near the Spirulina's cell (Fig. 6a), which invaded the cell 24 hours later (Fig. 6b) and began to destroy the membrane. Destruction of Spirulina cells has been caused by addition of 100 ppm concentration of Cu^{2+}-ions. Disruption of the cell membrane is clearly visible on the Figure 6c.

The investigation of ultrastructural changes of Spirulina cells after 5 days of incubation in a copper solution shows that the metal penetrates into the vacuole of cell in five days and, probably after conjugation with glutathione, is collected there (Fig. 6d). On Figure the presence of metal in the vacuole can be recognized. The analysis of obtained photos of 5 days has shown that the *Arthrospira platensis* has a high receptivity to a toxicant such as copper and so, this does not affect the viability of the cell itself (Tabagari et al. 2020).

At this stage, the initial data on Spirulina as the ecological potential of Spirulina are presented. The experiments continue and hope to find out the cause of some and interesting action of Spirulina, as a potential biosorbent for the purification of waters contaminated with heavy metals (Fig. 6).

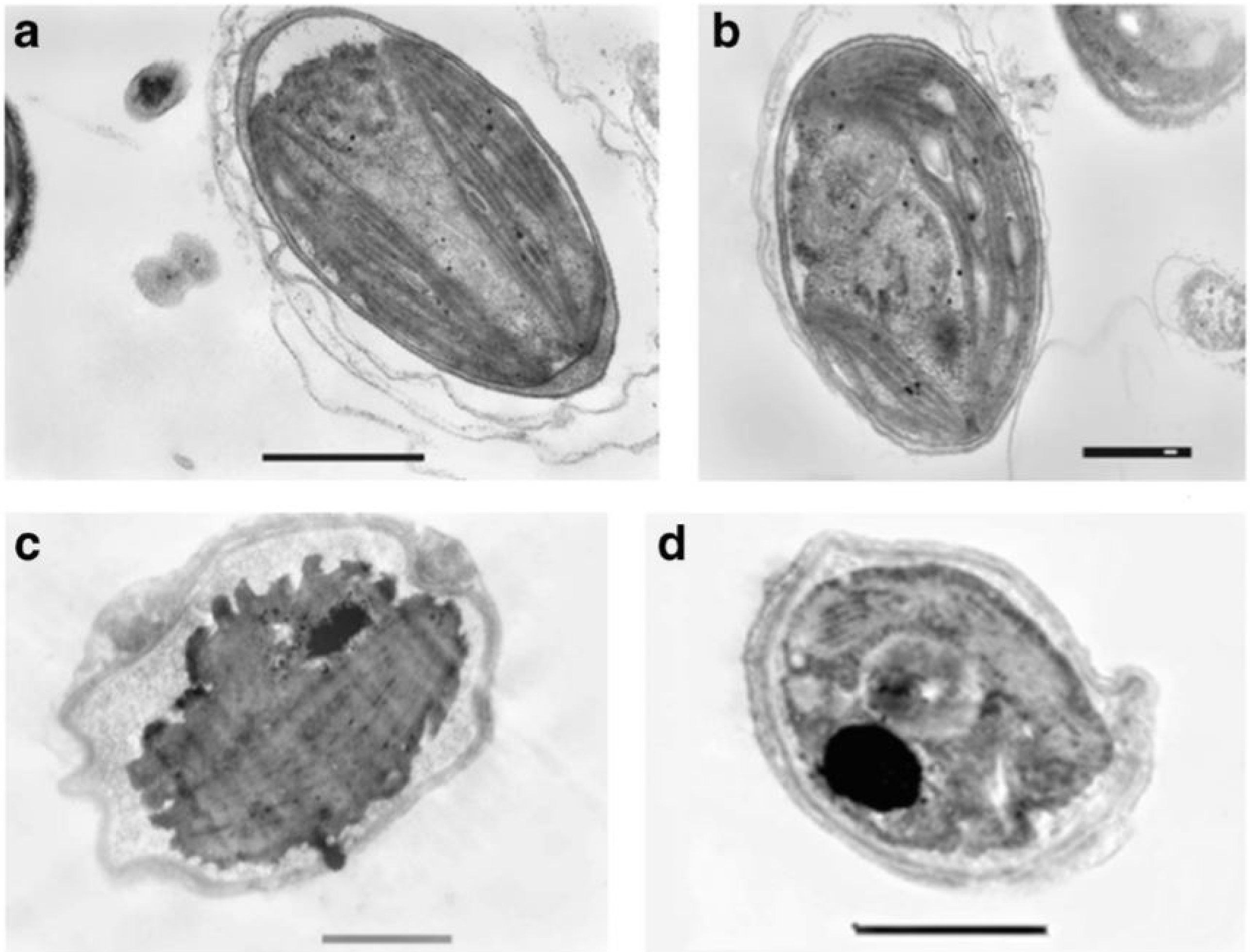

Fig. 6. Electron micrographs of Spirulina's cell cultivated on Cu^{2+} containing (100 mg/L) on standard Zarrouk's medium

a) Spirulina's cell on the 1st day—On the micrograph shows location of heavy metal near the Spirulina's cell. **b)** Spirulina's cell on the 2nd day—Copper enters into the cell. **c)** Spirulina's cell on the 3rd day—On the micrograph shows destruction membrane of Spirulina cells. **d)** Spirulina's cell on the 5th day—Collected heavy metals in vacuole.

4.5. The influence of Cu^{2+} and Pb^{2+} ions on the formation of biomass and chlorophyll of Spirulina

The third stage of the teats started in September 2019 and we started the experiments on lead-contaminated water. At the same time, were repeated the tests on the already tested copper in order to test and compare the absorption capacity of Spirulina with these two specific metals under the same conditions.

The effects of copper and lead on the biomass production of Spirulina compared to both 50 ppm concentration (Fig. 7a) and 100 ppm concentration (Fig. 7b) in polluted water.

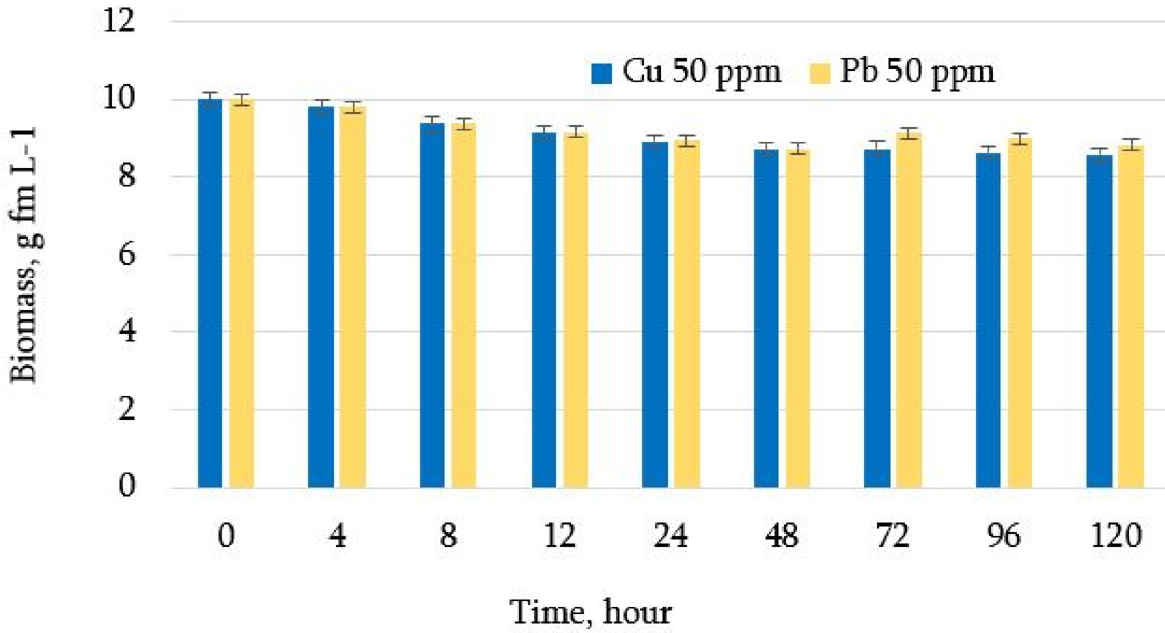

Fig. 7a. The influence of Cu^{2+} and Pb^{2+} ions (50ppm) on the formation of biomass of *Spirulina platensis* [p = 0.041]

Spirulina biomass decreased by 18% in the copper-contaminated incubation area as well as in the lead-contaminated area after 48 hours.

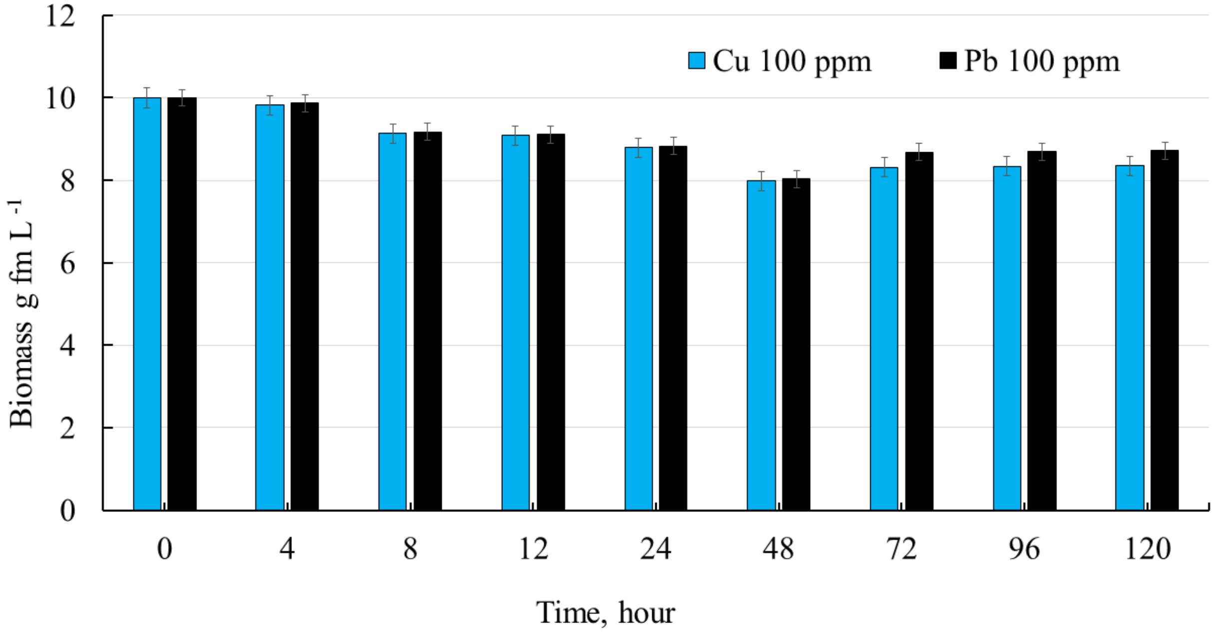

Fig. 7b. The influence of Cu^{2+} and Pb^{2+} ions (100ppm) on the formation of biomass of *Spirulina platensis* [p = 0.038]

Spirulina biomass decreased by 20% in the copper-contaminated incubation area as well as in the lead-contaminated area after 48 hours.

The effects of copper and lead on the chlorophyll accumulation of Spirulina was compared to both 50 ppm concentration (Fig. 8a) and 100 ppm concentration (Fig. 8b) in polluted water.

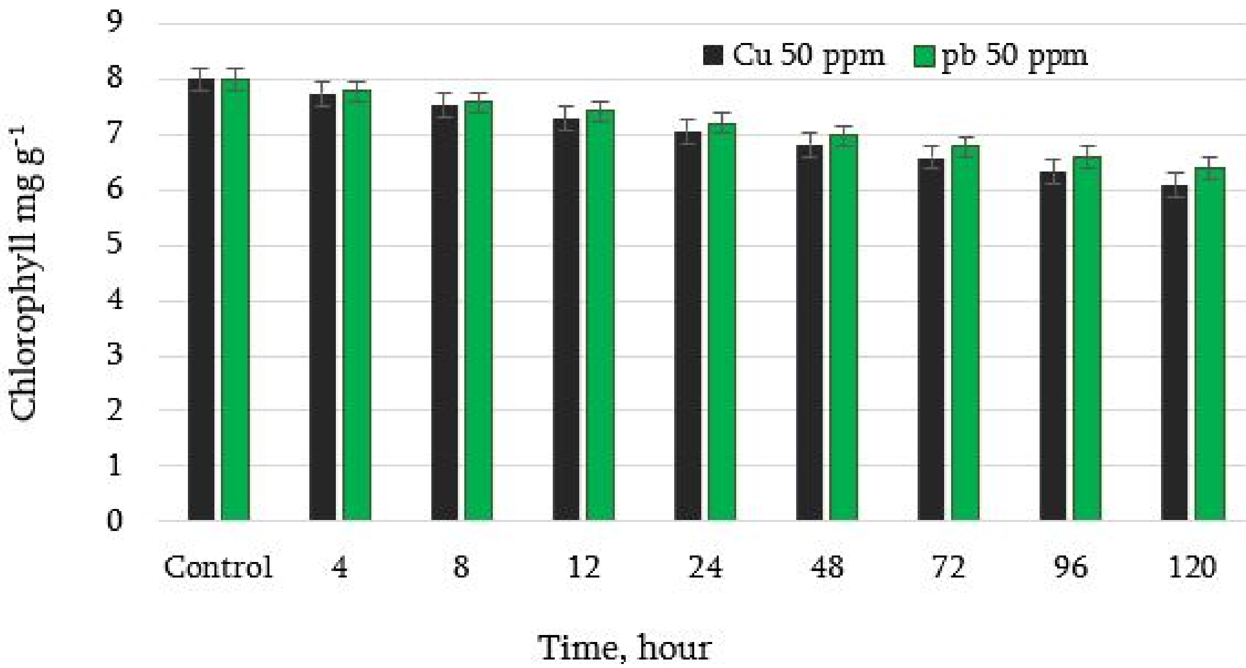

Fig. 8a. The influence of Cu^{2+} and Pb^{2+} ions (50ppm) on the formation of Chlorophyll of *Spirulina platensis* [p = 0.044]

Chlorophyll production was reduced by 23% in the contaminated water with both metals (separately) after 72 hours.

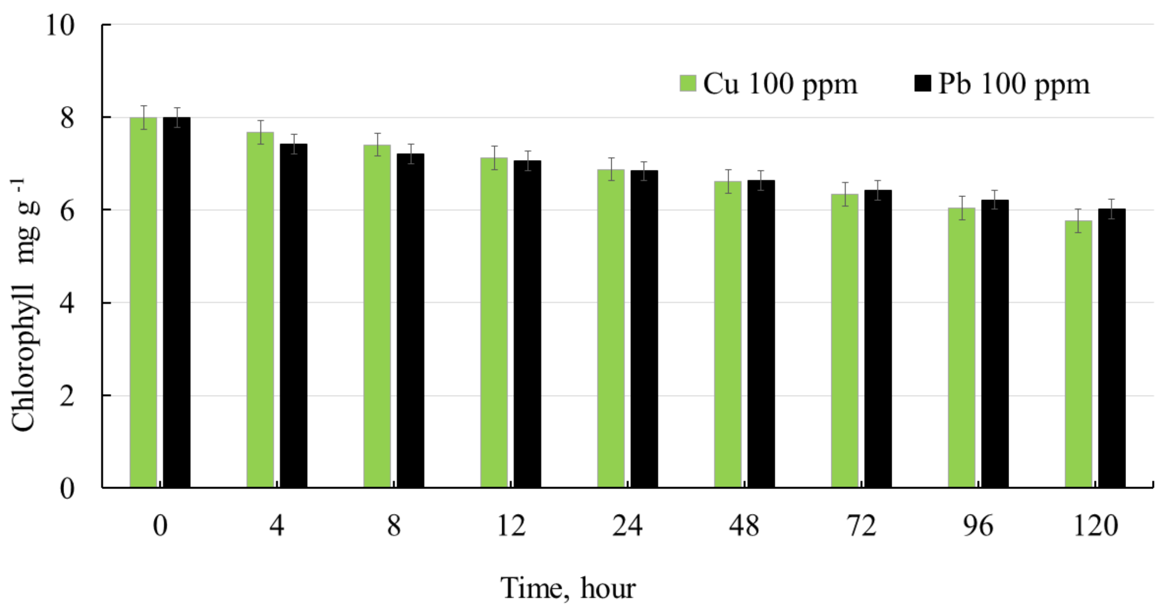

Fig. 8b. The influence of Cu^{2+} and Pb^{2+} ions (100ppm) on the formation of Chlorophyll of *Spirulina platensis* [p = 0.047]

Chlorophyll production was reduced by 25% in the copper-contaminated incubation area and in the lead-contaminated area after 72 hours.

The diagrams shown on Fig.8a and Fig.8b illustrate heavy metals are not an obstacle to the biological production of chlorophyll. In 100 ppm copper solution, chlorophyll formation is suppressed by only 25%. As a result, the process of photosynthesis lasts since heavy metals (copper and lead) do not destroy it. Absorb solar energy and its transformation into the chemical energy of organic substances continue. As observed, there are no significant changes that create chlorophyll.

As a result, Spirulina will exhibit its remedial properties in 100 ppm polluted water. As observed on Fig. 7b and Fig. 8b Copper and Lead cannot inhibit the main physiological indicator (biomass) of the developed *Arthrospira platensis.*

Model experiments to test Spirulina as a reducing agent for the purification of water contaminated with 100 ppm Cu^{2+} ions showed that the best results were obtained with 3.5 and 4.0 g/L of Spirulina biomass (Table 1 and Table 2). As can be seen from the data obtained, in these cases the reclamation efficiency was reached up to 70 %. When the concentration of Cu^{2+} and Pb^{2+} ions and the initial Spirulina biomass were increased, the absorption efficiency was only about 25%. These results show that the most effective ratio of heavy metal to untreated algae biomass was approximately 1:35 (Table 1).

The obtained results show that maximal assimilation by Spirulina is 79 % in case of Cu^{2+}ions and 85% in case of Pb^{2+} ions after incubation at 100 ppm concentration of heavy metal. Here it should be noted that the maximum period of incubation of Spirulina in contaminated copper in the medium was 5 days and this is not accidental. It turns out that Spirulina reveals the physicochemical absorption of the metal at the initial stage of incubation and rather quickly. According to literature data, other researchers also received a similar effect. Following their experiences absorption of copper occurs very quickly. Most of the metal is absorbed during the first 15-30 minutes. At the same time, growing conditions do not affect the dynamics of metal absorption. Spirulina is absorbed mainly due to ion exchange and, to a lesser extent, physical adsorption (Markou et al. 2015). Naturally, the question arises of how the process of absorption occurs in dynamics, and as it turned out by us, after a 3-day incubation, the metal gradually begins to return to the incubation medium. At this stage of our research, we can only assume that due to the osmotic gradient, an excess amount of metal is returned to the solution. In the future, we are going to find out the reason for such an interesting effect of the absorption-resorption process, as well as the similar behavior of Spirulina should be taken into account in the technical regulations for wastewater treatment from heavy metals and it is obvious that multistage wastewater treatment from heavy metals should be based on the speed and effectiveness of Spirulina, as a remediation tool, at a particularly initial stage of the cleaning process.

As it seen from the results, no significant change was observed in the physiological parameters, although 100 ppm was more informative and it was decided to continue the following experiments at this dose.

4.6. Study of the dynamics of Cu^{2+} and Pb^{2+} ions uptake by Spirulina cells (by Atomic adsorption spectrophotometry)

In the early stages of the experiments, the cleaning efficiency of Spirulina of contaminated water with heavy metals was determined using a photoelectric colorimeter in the incubation area. Due to the tests determined by UV-visible Spectrophotometer heavy metal contaminated water was purified from 79 to 85% of copper and lead, respectively, it was of great interest to determine the amount of heavy metals entering the biomass itself.

The number of metals absorbed by the algae was determined by atomic absorption spectroscopy. As shown in diagrams (Fig.9), in the control samples, the heavy metals existing in the pure Spirulina biomass were equal to 0.

Strange as it may seem, but lead ions were absorbed 3.5 times more quantities than copper ions. As a result, maximal absorption of lead by *Spirulina platensis* was 71ppm, and maximum amount of Cu^{2+} ions absorbed by 1 g fresh biomass of Spirulina is 19 ppm in 72 hours.

As the data shows, for both metals, maximum absorb was observed at 72 hours for 5 days.

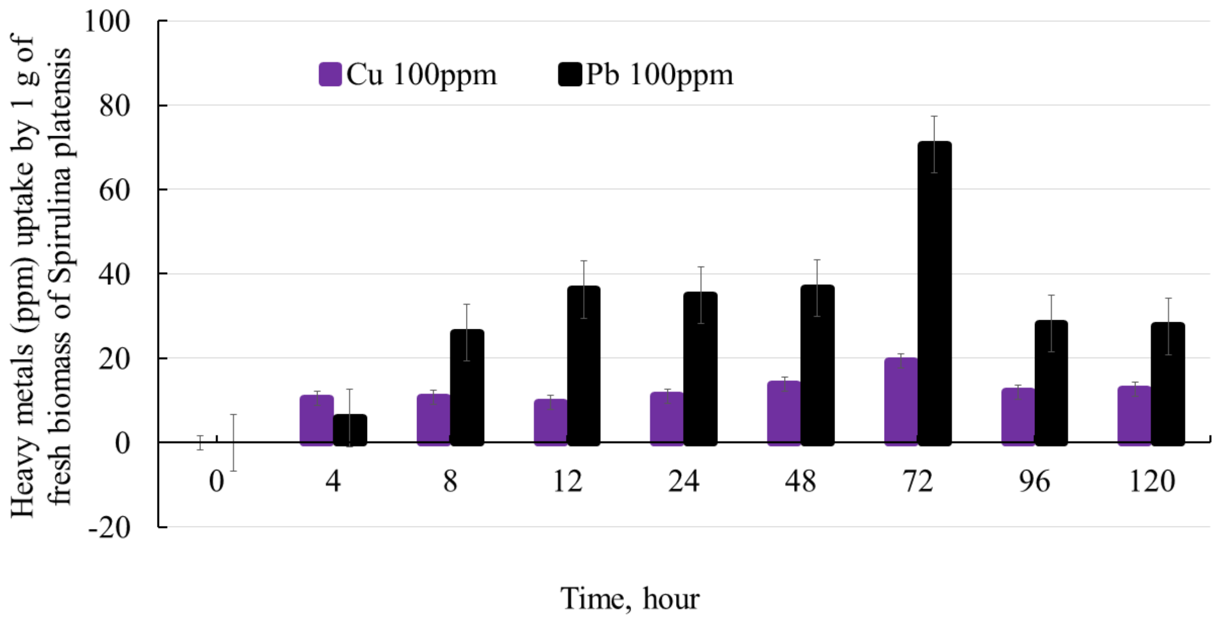

Fig. 9. Heavy metals absorbed by Spirulina, by Atomic Absorption Spectrometer [p = 0.035]
The maximum amount of Cu^{2+} ions absorbed by 1 g fresh biomass of Spirulina is 19 ppm in 72 hours. With Pb^{2+} ions, the maximum absorption is 71ppm in 72 hours. The controls had a copper and lead content of 0.

4.7. Effect of EDTA on the absorption of heavy metals (Cu^{2+} and Pb^{2+}) by Spirulina during the 120 hours

The classic chelating agent EDTA used to enhance remediation potential of *Spirulina platensis*, Increased absorption at 4, 8, and 24 hours, copper-contaminated water (Fig. 10a). As for lead-contaminated water, the peak shifted from 72 hours to 48 hours, and according to 4-hour data, the maximum amount of lead ions absorbed increased 9-times after the addition of EDTA (Fig. 10b).

Since adding EDTA, the results show that the ability to absorb Spirulina is increased in the first 8 hours for both metals. However, the maximum amount of absorbed metal differs between copper and lead samples. From a copper-contaminated environment, it boosted by 63%. In contrast, it decreased with lead by 10%. Thus, EDTA only helped speed up the penetration process (Fig. 10a and Fig. 10b).

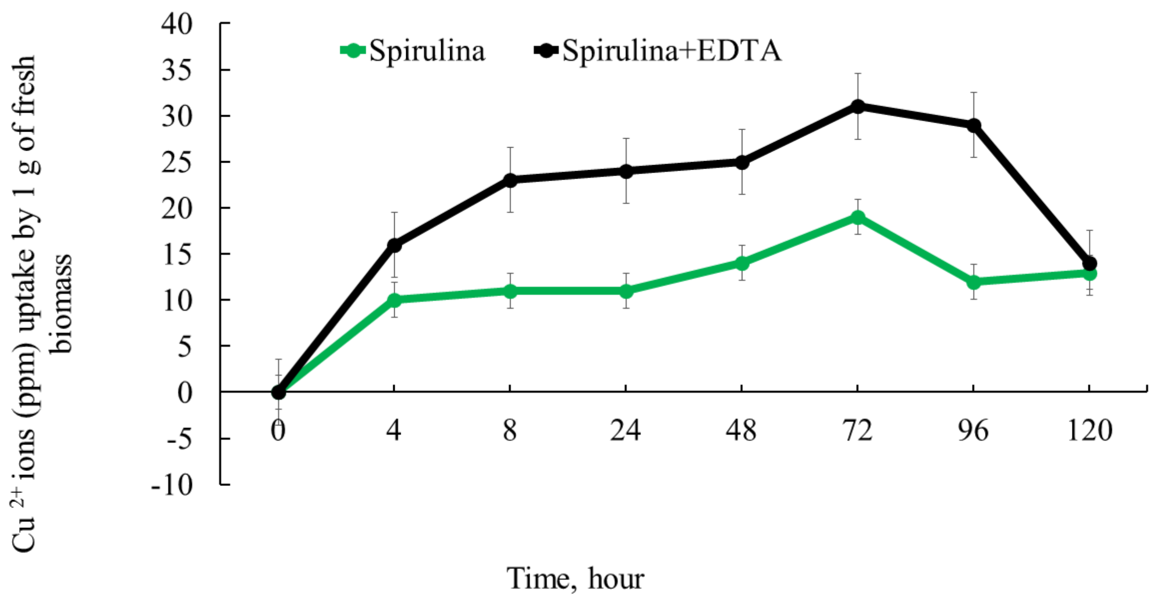

Fig. 10a. Effect of EDTA on Cu^{2+} ions uptake by *Spirulina platensis* [p = 0.033]
EDTA increased the amount of copper absorbed by Spirulina by 63%. The copper content in both controls were 0.

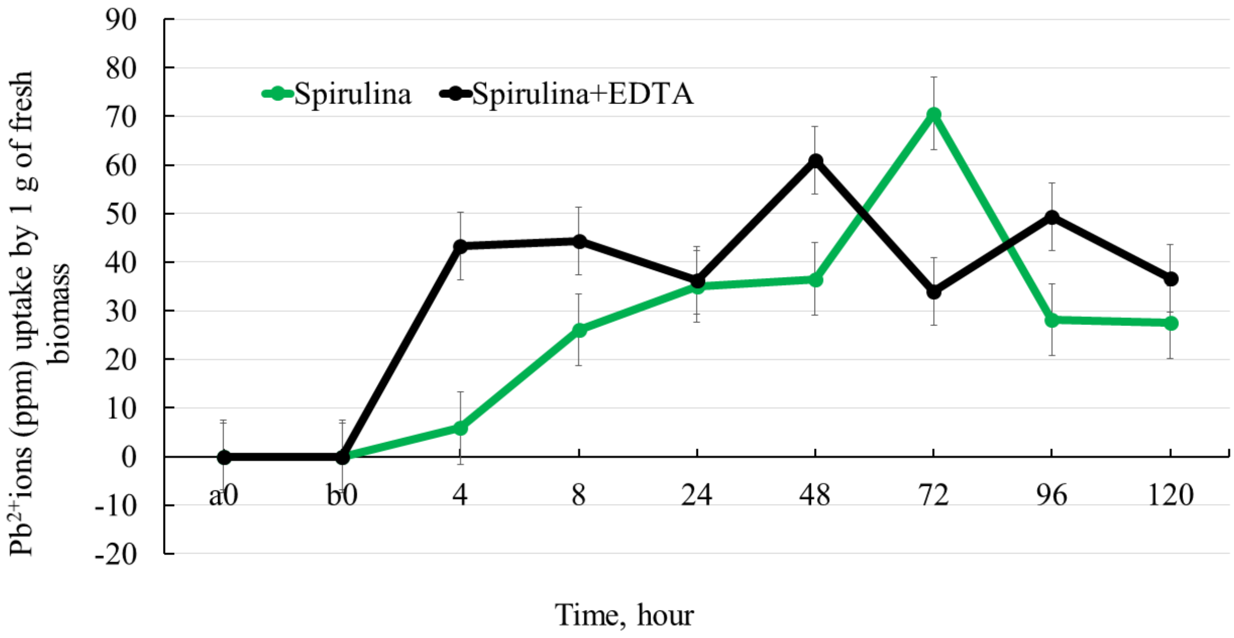

Fig. 10b. Effect of EDTA on Pb^{2+} ions uptake by *Spirulina platensis* [p = 0.045]
The maximum amount absorbed by Spirulina is 10% higher than by Spirulina in the consortium with EDTA. The lead content in both controls were 0.

It can be assumed that chelating copper with EDTA is more easily absorbed by Spirulina than lead. Probably, the fact that lead is slightly more than 3 times heavier than copper plays an important role in this respect. It was interesting for us whether the same trend would show up in the difference between copper and lead in the case of biosurfactants.

4.8. Results of absorption of Cu^{2+} and Pb^{2+} ions with the combined action of Spirulina and biosurfactants

According to the results, the lipid Rhamnolipid1, Rhamnolipid2, and Trehalose showed unique properties in combination with Spirulina for both metals (Fig. 11a and Fig. 11b). As shown in the comparable graphs, RL1 accelerated the absorption process during the first 8 hours. RL2 shifted the absorption peak from 72 hours to 48 hours for lead contamination. But with copper, the peak remains for 72 hours. However, the maximum amount of absorbed metals was increased by copper, not lead. The Trehalose lipid showed better results than both rhamnolipids in absorbing heavy metals by *Spirulina platensis*, which will be taken into account in process of creating a wastewater treatment technology. Biosurfactants activate the remedial abilities of Spirulina – particularly, RL1 increased the maximum amount of absorbed Cu^{2+} ions by 42%, RL2 increased the maximum amount of absorbed Cu^{2+} ions by 68%, and TRL by 73%. The action of biosurfactants is even more important because of their environmental friendliness.

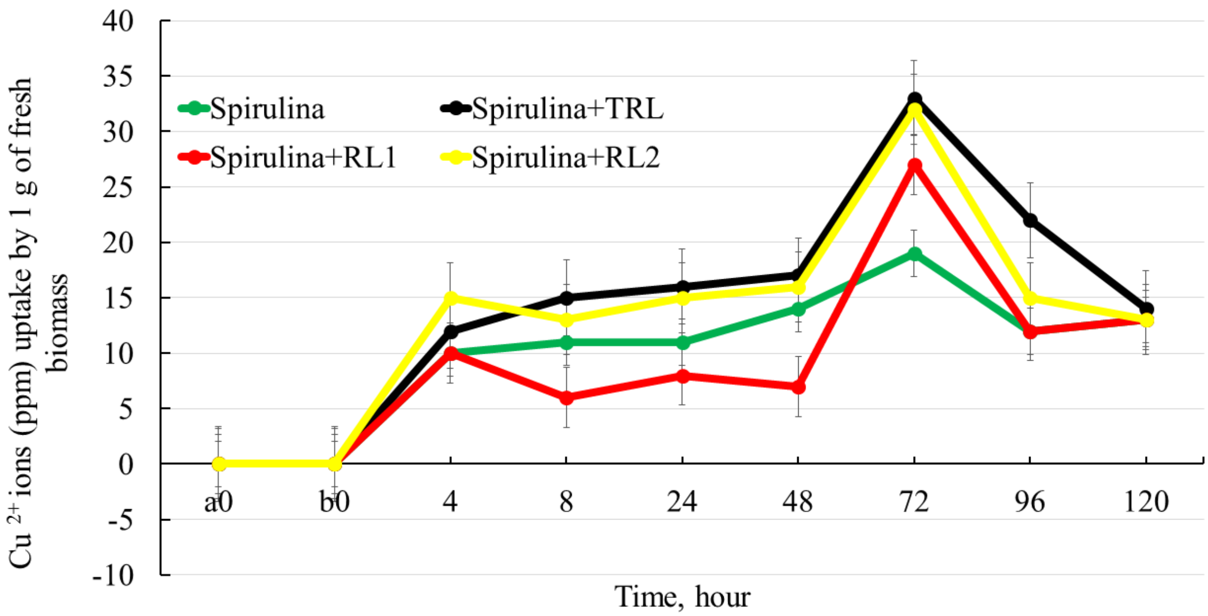

Fig. 11a. Comparative analysis of biosurfactants RL1, RL2 and TRL on the absorption capacity of Cu^{2+} ions by *Spirulina platensis* [p = 0.031]

RL1 increased the amount of copper absorbed by Spirulina by 42%. RL2 increased by 68%, and TRL by 73%. The copper content in controls were 0.

In bioremediation, scientists have studied different methods to improve the remediation skills of Spirulina. For that reason, some add carbohydrates to increase the number of absorbed metals (Markou et al. 2015). Others studied the intake capacity of dry and raw Spirulina and discovered that dry was more skillful at purifying water than raw one (Al-Homaidan et al. 2014). However, the increase picked up in both investigations does not exceed 5%. Still, we involved improving the absorption of heavy metals by algae in less time. And the amount of absorbed copper increased by 73% with Trehalose lipid.

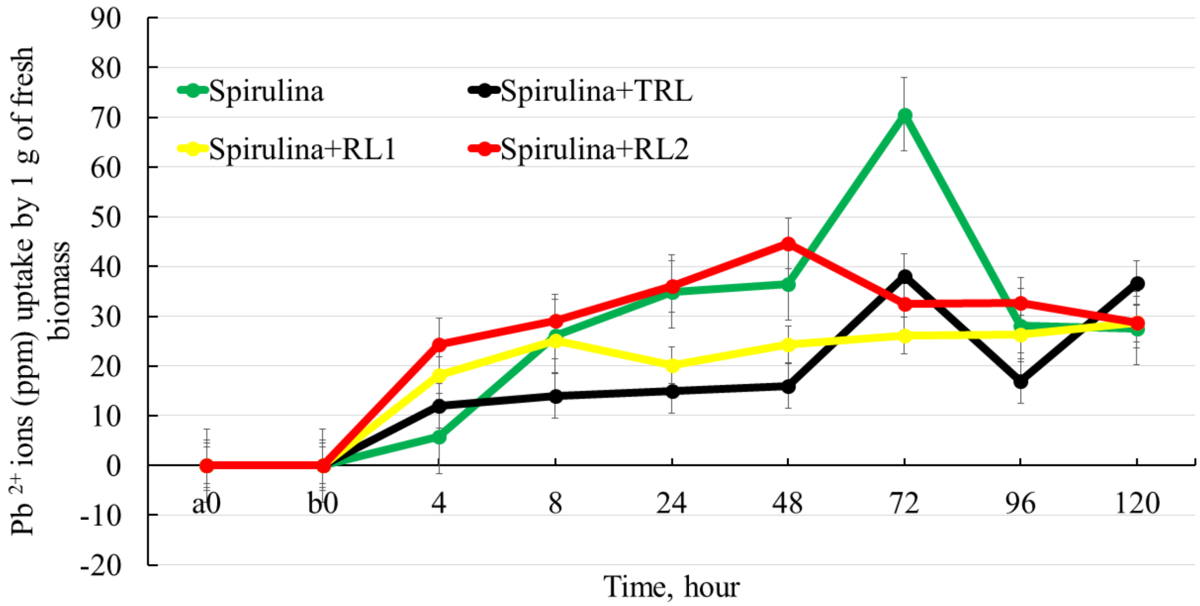

Fig. 11b. Comparative analysis of biosurfactants RL1, RL2 and TRL on the absorption capacity of Pb^{2+} ions by *Spirulina platensis* [p = 0.038]

The maximum amount of lead ions absorbed by Spirulina is 72%, Spirulina in consortium with RL1 – 24%, with RL2 – 32%, and with TRL – 39%. The lead content in controls were 0.

As the results show, Spirulina absorbs lead ions 3 times less in combination with RL1, 2.3 times less in combination with RL2, and 1.8 times less in combination with TRL than without additives. Since Spirulina absorbs more lead ions without any additives, it is likely that lead, together with chelators, forms a larger complex that is too difficult to absorb by algae.

Here, should be noted – after reaching the maximum concentration of metal in Spirulina biomass – at 72 hours, the metal returns to the incubation medium. This requires further research. At this stage, one can only assume that this may be because of the osmotic gradient. Of course, this behavior of *Arthrospira platensis* must be considered in remediation methods against metals. The release of metal from Spirulina into the solution after 72 hours of incubation occurs less in experiments with the EDTA.

The advantages of Spirulina are that it can multiply in salty and even alkaline environments, which are a barrier to other microorganisms. Still, many studies prove the ability of various microorganisms to absorb heavy metals and their apparent ability to purify water. However, Spirulina is the cheapest, healthiest and friendliest product, as its medicinal properties were researched many centuries ago.

5. Conclusion and Recommendations

The experimental results of the presented work can be the basis for the development of phytoremediation technology, which will be based on the use of *Arthrospira platensis* to treat water contaminated with target toxins. The main idea of these technologies is to add Spirulina biomass to water contaminated with Cu^{2+} and Pb^{2+} ions and to periodically remove it from the water surface (or filter it) as soon as the remediation process is completed. The effective time of absorption of metal ions by Spirulina biomass should also be taken into account, as well as the nuance that after saturation of Spirulina cells with metal ions, metal ions are released back into the incubation solution. Probably the reason for this must be the difference in the osmotic gradient between the Spirulina cells and the incubation area.
It is for this reason that the metal-containing fluid in the remediation reservoir should be delayed for only 2-3 days, which coincides with the maximum effective absorption of Spirulina.

The presented paper explains the role of biosurfactants in increasing the remedial capacity of Spirulina, i.e. in improving the sorption properties of Spirulina in an area contaminated with copper and lead ions. The obtained experimental data show the effectiveness of their joint action.

Particularly, the obtained results show that:

- **The optimal amount of sodium bicarbonate for high productivity of Spirulina biomass was chosen - 16.8 g. for 1 liter of Zarrouk's medium;**

- The Spirulina biomass in the Zarrouk's medium increased 2 times better than in the area of physiological solution;
- The chlorophyll accumulation 20% lower in the salted water then Zarrouk's area;
- 100 ppm concentration of Cu^{2+}-ions slightly affects the homeostasis of this biosystem which causes the inhibition of biomass accumulation by 15%, and the decrease of chlorophyll content by 30%.
- Spirulina biomass decreased by 18% in the lead-contaminated area after 48 hours;
- Chlorophyll production was reduced by 23% in the lead-contaminated water after 72 hours.
- High concentrations of glutathione significantly reduce the content of free Cu^{2+} in the incubation area that clearly indicates participation of glutathione S-transferase in Cu^{2+} salvaging process by Spirulina cells;
- The degree of cleaning was 79%, in the case of 100ppm concentration of Cu^{2+} ions and 4.5 g/L initial content of *Arthrospira;*
- The degree of cleaning was 85%, in the case of 100ppm concentration of Pb^{2+} ions and 4.5 g/L initial content of *Arthrospira;*
- The micrograph clearly shows the location of heavy metal invaded the cell 24 hours later and began to destroy the membrane;
- After 5 days of incubation in a copper penetrates into the vacuole of the cell in five days;
- **The maximal assimilation by Spirulina is 79 % in case of Cu^{2+}ions and 85% in case of Pb^{2+} ions after incubation at 100 ppm concentration of heavy metal.**
- EDTA increased the amount of copper absorbed by Spirulina by 63%;
- **RL1 increased the amount of copper absorbed by Spirulina by 42%.**
- **RL2 increased the amount of copper absorbed by Spirulina by 68%,**
- **TRL increased of copper absorbed by Spirulina by 73%.**
- **RL1 reduced the amount of lead absorbed by Spirulina by 3 times;**
- **RL2 reduced the amount of lead absorbed by Spirulina by 2.3 times;**
- **TRL reduced the amount of lead absorbed by Spirulina by 1.8 times;**

- from the lead-contaminated water, the chelating agents only sped up the absorption process.
- Accumulated heavy metal ions in Spirulina cells come back into the water, so the alga biomass has to be separated from the water after a day from the start of incubation.

Thereby, based on the results of experiments, it was proved that the capacity of Spirulina to phytoremediation demonstrates that *Arthrospira* algae have the ability to effectively purify water contaminated with copper ions, which can be based development of modern technologies of purification of wastewater.

This thesis can provide a cheap and friendly approach to purifying wastewater from copper and lead ions.

Further studies will include an ultrastructural analysis of Spirulina and reveal the mechanisms of sorption and desorption of metals by Spirulina more accurately also Mass-spectrometry provides important information that leads to the identification and quantification of the individual capabilities of biosurfactants – how they can improve the penetration of heavy metals into the *Spirulina platensis*. As a result, the basic idea of mechanisms for improving and accelerating the absorption properties of heavy metals by biomass *Arthrospira platensis* with the addition of ecological additives-biosurfactants was implemented with a reasonable result.

Detailed data are described in the articles I have published:

Tabagari I, Kurashvili M, Varazi T, Adamia G, Gigolashvili G, Pruidze M, Chokheli L, Khatisashvili G, Niemsdorff PVF (2019) *Application of Arthrospira (Spirulina) platensis against chemical pollution of water*. **Journal Water**, MDPI 11:1759-1768. https://doi.org/10.3390/w11091759

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