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ORIGINAL ARTICLE

Investigation of Lindane (Γ-Hexachlorocyclohexane) Destruction in Saline Soil by Local Cyanobacteria Strains

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ABSTRACT
Cyanobacteria, due to photosynthetic products (exudates) and ability to fix atmospheric nitrogen, can serve as a precious base in the creation of a nitrogen-fixing ecosystem. They are also particularly resistant to pollutants and toxic substances. Cyanobacteria carry out complete or partial degradation of complex organic compounds, including herbicides, pesticides, and petroleum products. It was established that the studied strains of cyanobacteria Nostoc linckia 4, N. muscorum 14, N. pruniforme 20, Gloeothece rupestris 15, Synechococcus cedrorum 12 and their associations (N.pruniforme 20 + Gloeothece rupestris 15) demonstrated very high and stable HCH-destructive activity during 4 months under salt stress (4% NaCl). The γ-hexachlorocyclohexane (HCH – commonly used pesticide) content decreased significantly from 2 µg/g soil to 14 ng/g soil, which is less than 1% of the introduced HCH. It was shown that under salt stress conditions (300 mM, NaCl) the studied cyanobacterial strains produced exopolysaccharides (EPS). It was found that when cyanobacteria N. linckia 4, N. muscorum 14, N. pruniforme 20 and Gl. rupestris 15 were cultivated for 7 days at a sodium chloride concentration of 300 mM, EPS production increased compared to the control by 25.5%; 23.6%; 16% and 15.1% respectively. Additionally, under salt stress conditions, local strains of cyanobacteria exhibited the activity of the ACC deaminase enzyme. Thus, after 14 days of cultivation, N. pruniforme 20 and Gl. rupestris 15 cultures produced 94 and 85 µmol/mg protein/h in the control variant, while under salt stress conditions the enzyme activity increased by 21.3 % and 27%, respectively. The results of presented study demonstrated that local strains of cyanobacteria can be effectively used for protection of the crops by elimination of salinity and pesticide related hazards.
Key words: *cyanobacteria, γ-hexachlorocyclohexane, destruction, saline soil, pesticides, exopolysaccharides, ACC deaminase*

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INTRODUCTION

Modern intensive agriculture depends on the use of various agrochemicals (herbicides, insecticides, fungicides, etc.). Pesticides are a group of chemicals used to kill insect pests, weeds, fungi, bacteria, etc. Organochlorine pesticides are synthetic pesticides that are widely used in the chemical industry and agriculture. These compounds have the following properties: high toxicity, persistence, slow degradation, and bioaccumulation [1]. Even though the use of these agents has been banned in developed countries, in practice there is an abuse of these chemicals and currently the consumption of pesticides reaches more than two million tons around the world [2]. It has been established that chemical compounds present in the environment - in air, water, food, and soil - have a variety of harmful effects on human health [3]. A study of the effects of various classes of pesticides leads to the conclusion that many of them are responsible for hypertension, cardiovascular diseases and other diseases in humans [4]. Cyanobacteria are the oldest photosynthetic organisms on earth, the appearance of which dates back to about 2.6-3.5 billion years ago [5]. Cyanobacteria are morphologically diverse and exist in different forms, unicellular, filamentous, and colonial (coccoid) in planktonic or benthic communities [6]. Cyanobacteria are ubiquitous and exceptionally well adapted to a wide range of environmental conditions [7-9]. They are

considered pioneering organisms which occupy new territories after environmental stresses (eruption of volcanos, fires, salinization of soils, pollution by pesticides and herbicides, etc.) [10-11]. The role of cyanobacteria (blue-green algae) in biocenoses developing under extreme conditions is especially important as an accumulator of organic matter, a molecular nitrogen fixer and a stimulator of soil microbiological activity [9,12,13]. Cyanobacteria possess special resistance against polluting and toxic substances. They carry out full or partial degradation of complex organic compounds, including herbicides, pesticides, mineral oil [14,15].

γ-Hexachlorocyclohexane (lindane) has historically been used as a broad-spectrum pesticide in agriculture, livestock, forestry, and veterinary medicine. Several harmful factors have led to concerns about the production and use of lindane, including its persistence, toxicity, bioaccumulation, and long-range transport potential. Over the past two decades, great attention has been paid to the destruction and cleanup of the environment contaminated with this dangerous material. A number of methods have been developed for the elimination and purification of lindane from soil and water, such as activated carbon sorption, nanoparticle reduction, photocatalysis, biocatalytic dechlorination, phytocorrection, biosorption and microbial degradation. Among all these above methods, microbial degradation of these toxicants is cheaper, environmentally friendly, and effective for removing them from the environment [16,17].

In the world, the annual reduction in the amount of areas suitable for planting agricultural crops requires the introduction of intensive biological methods, the use of which will increase soil fertility and reduce the cost of agricultural products. In this regard, cyanobacteria, which have a wide range of biologically valuable properties, play a key role in the global nitrogen cycle and maintaining a stable ecosystem in extreme conditions. They can be involved in measures to improve soil fertility and increase the productivity of agricultural products [18,19].

In the conditions of Uzbekistan, the degradation of organochlorine pesticides has been studied little, which makes the study of the issue of biodegradation of organochlorine pollutants very relevant. Of particular concern is the accumulation in environmental objects of residues of various chloroaromatic compounds and, first of all, chlorophenols, which can form polychlorinated structures with carcinogenic properties. The danger of such organic pollutants is aggravated by their high persistence to photolytic, chemical, and biological decomposition.

Based on the above, the purpose of our research was to study the degradation of γ-hexachlorocyclohexane (lindane) by local salt-tolerant strains of cyanobacteria isolated from saline soils of Uzbekistan as well as the production of EPS and ACC deaminase under salt stress conditions. In such a situation, soil microorganisms with ACC deaminase enzyme activity break down ACC into α-ketobutyrate and ammonia, helping in reduction of ethylene production, which inhibits the plant growth. As a result, it becomes easier for plants to adapt to stressful conditions and survive better with enhanced growth and productivity [20].

MATERIAL AND METHODS

Objects of research

The objects of the study were algal and bacterially pure cultures of local strains of cyanobacteria: *Nostoc linckia* 4*, N. muscorum* 14*, N. pruniforme* 20*, Gloeothece rupestris* 15, *Synechococcus cedrorum* 12 obtained from the collection of the Institute of Microbiology isolated from saline and polluted pesticides of soils in Kashkadarya, Syrdarya and Namangan regions of Uzbekistan [21,22].

Cyanobacteria cultivation

For the cultivation of cyanobacteria, liquid medium BG-11 without nitrogen was used [23]. The liquid medium was poured into flasks with a volume of 250-300 ml so that the occupied volume was not more than 1/3 - 1/4 of the volume of the flask. The vessels with the seeded material were placed in light with fluorescent lamps, at 2.5-3 thousand lux, at a temperature of 27-28^{0}C.

Determination of HCH degradation by cyanobacterial strains in model experiments in soil

Aliquots of standard sierozem soil 50 g were sterilized by heating in thermostat at 180^{0}C for 4 hours. We introduced 0,1 mg hexachlorocyclohexane (10 ml) in each Petri dishes, containing 50 g of dry sterile soil sample with 4% NaCl. Initial concentration of hexachlorocyclohexane (HCH) in the soil sample was 2 μg/g of soil. Then soil samples were dried up to full evaporation of HCH. After that we added on each sample 8 ml suspension of cyanobacteria (humidity of 60%), mixed, closed by cover and left at room temperature. After month of incubation, we selected 1 g of dry soil, extracted HCH by hexane and analyzed by gas chromatography on chromatograph with electron capture detector Agilent Technologies 6890N. Conditions of chromatography: capillary column HP5, length 30 m, diameter 0,25 mm, stabile liquid phase - 5% phenylmethyl silicone. Chromatography was carried out by RTL method. Temperature of evaporator = 250oC, oven temperature = 70 ^{o}C for 2 min, further rise of temperature up to 150 ^{o}C with

rate 25 °C/min. After that rate of temperature up to 200 °C with rate 30 °C/min. After that rise of temperature up to 280 °C with a speed 8 °C/min. After last rise of temperature - 10 minutes at 280 °C. Pressure in column = 12,21 psi. The gas - carrier - nitrogen. The detector - μECD, temperature of detector = 300 °C.

Quantitative analysis of hexachlorocyclohexane (lindane) was carried out with calibration curve in software Agilent ChemStation of chromatograph Agilent Technologies 6890N by method of external standard (ESTD) and recalculated the received quantity on 1 g of soil.

Isolation of exopolysaccharides

To study the quantitative production of exopolysaccharides, cyanobacteria were cultivated in BG-11_0 medium in the presence of 300 mM sodium chloride for 10 days. Strains were collected from the nutrient medium after 7 and 14 days by centrifugation at 6000 rpm for 30 min. The culture fluid separated from the biomass was precipitated by adding ethanol and stored at 4 °C for 24 h. The precipitates were collected and placed in a drying cabinet to evaporate the remaining ethanol. Finally, the dry mass of the precipitate was determined relative to the control [24].

Determination of 1-aminocyclopropane-1-carboxylate (ACC) deaminase enzyme activity

Quantitative analysis:

ACC deaminase positive cyanobacteria were cultured in M9 broth supplemented with 3 mM ACC and incubated on a shaker (48 hours at 37°C, 150 min/rotation). Incubated samples were centrifuged in the cold (10,000 rpm for 10 min) and the supernatant was measured in a spectrophotometer at 540 nm. The amount of α-ketobutyrate produced by the cyanobacterial isolate after ACC degradation was determined based on the α-ketobutyrate standard graph [25].

Statistical Analysis

The statistical significance of data was tested by the analysis of variance of the Microsoft Excel 2010 package. Mean comparisons were conducted using the least significant difference test (p= 0.05). The average values of average values of microorganisms' growth and development parameters, EPS and ACC deaminase production and the standard deviation were counted based on three replications.

RESULTS AND DISCUSSION

In this work, we used local strains of cyanobacteria *Nostoc linckia* 4, *N. muscorum* 14, *N. pruniforme* 20, *Gloeothece rupestris* 15, *Synechococcus cedrorum* 12 isolated from saline soils contaminated with pesticides in various regions of our Republic (Fig. 1). We found that after month of incubation of soil samples with cultures of investigated strains of cyanobacteria *Nostoc linckia* 4*, N. muscorum* 14*, N. pruniforme* 20*, Gloeothece rupestris* 15 and association of cyanobacteria (*N. pruniforme 20 + Gloeothece rupestris* 15) the concentration of hexachlorocyclohexane has essentially decreased from 2 μg / g of soil to 14 ng/g of soil in comparison with control (Fig.2). Thus, in the soil samples with cyanobacteria the concentration of hexachlorocyclohexane (lindane) was less than 1% from introduced initially lindane, except only one strain *Synechococcus cedrorum* 12 for which decrease of quantity lindane was not so high and residual quantity of lindane was 40 % from introduced amount. Practically all investigated cyanobacteria strains had very high hexachlorocyclohexane-destructive activity in the saline soil with 4% sodium chloride. In the studies conducted, the association of cyanobacteria *N. pruniforme* 20 + *Gl. rupestris* 15 also actively degrade HCH under conditions of 4% salt stress. Investigated cyanobacteria strains and their association have shown very high and stable lindane degradation activity. Thus cyanobacteria *N. linckia* 4*, N. muscorum* 14*, Gl. rupestris* 15, and *N. pruniforme* 20 actively downgraded introduced lindane, and residual quantity of hexachlorocyclohexane was 14.34501 ng / g of soil, 14.36717 ng / g of soil, 14.20315 ng / g of soil and 14.21454, accordingly (Table 1).

It was also found that associations of cyanobacteria *N. pruniforme* 20 + *G. rupestris* 15 also actively degrade HCH under conditions of 4% salt stress. The data obtained give reason to believe that the studied local strains of cyanobacteria degrade or transform HCH to volatile compounds or completely utilize it. It should be noted that the concentration of introduced HCH in the samples was quite high – 2 μg / g of soil. Therefore, the observed almost complete destruction of HCH within 1 month under salinity conditions (4% NaCl) in the samples indicates enhanced HCH-degradation activity of local strains of cyanobacteria. Since HCH is almost completely decomposed by local strains of cyanobacteria, it is likely that when cyanobacteria are incubated with HCH, enzymes involved in the down braking of pesticides such as polyphenol oxidase, phenol oxidase, catalase, superoxide dismutase, etc. are induced [26]. It is known that among photoautotrophic organisms *Anabaena* sp. PCC 7120, *Nostoc ellipsosporum* and *Microcystis aeruginosa* PCC 7806 have been described as potential lindane degraders [27,28].

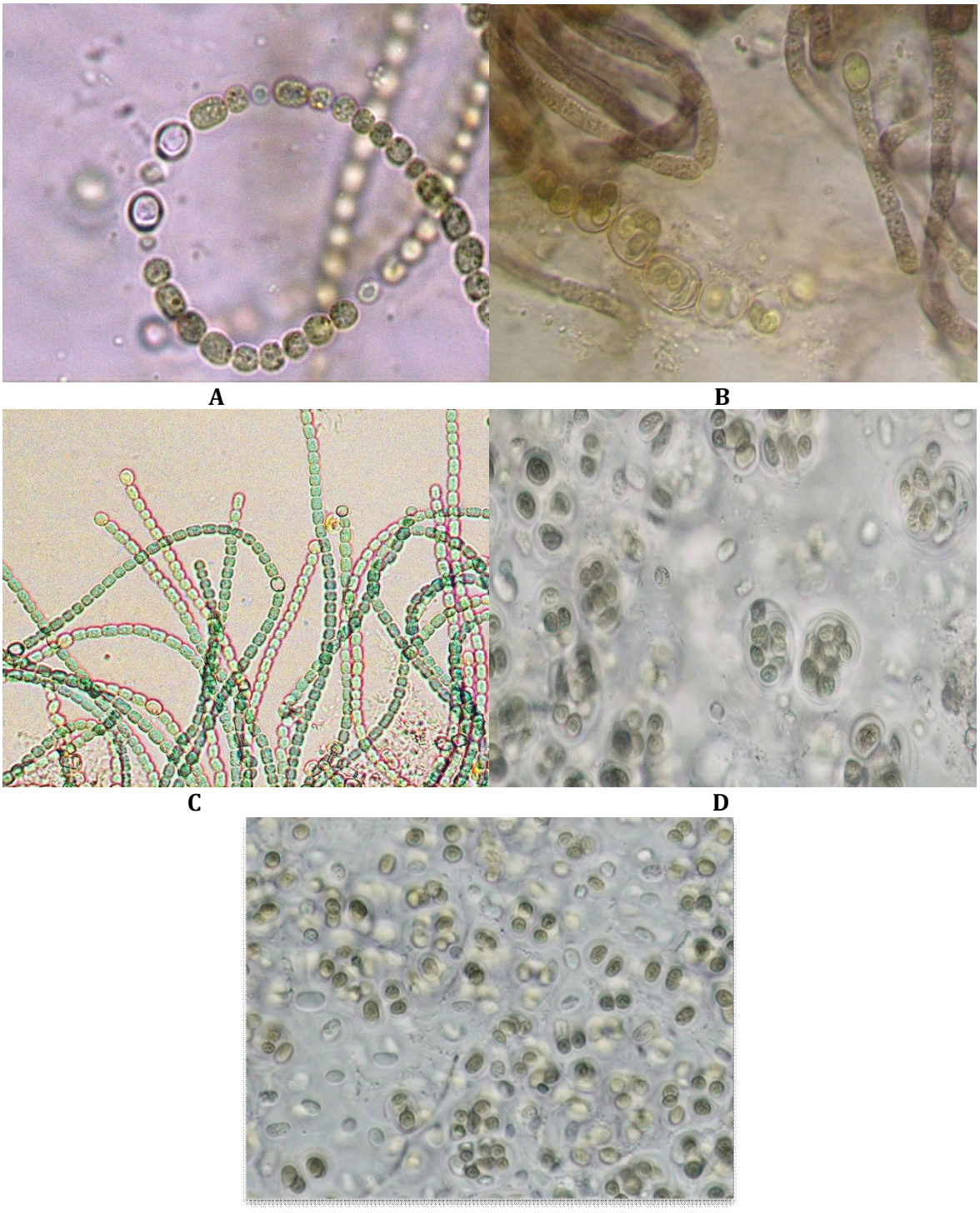
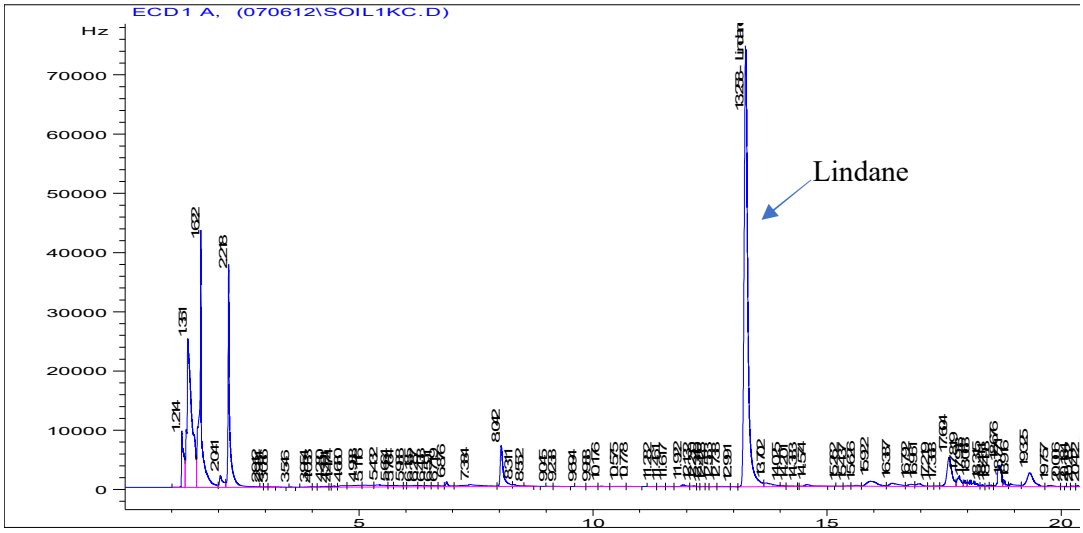


Figure 1. Microscopic photographs of cyanobacterial cells: a) Nostoc linckia 4; b) Nostoc muscorum 14; c) *Nostoc pruniforme* 20; d) Gloeothece rupestris 15; e) Synechococcus cedrorum 12 (Magnification: 100 x 13.5)



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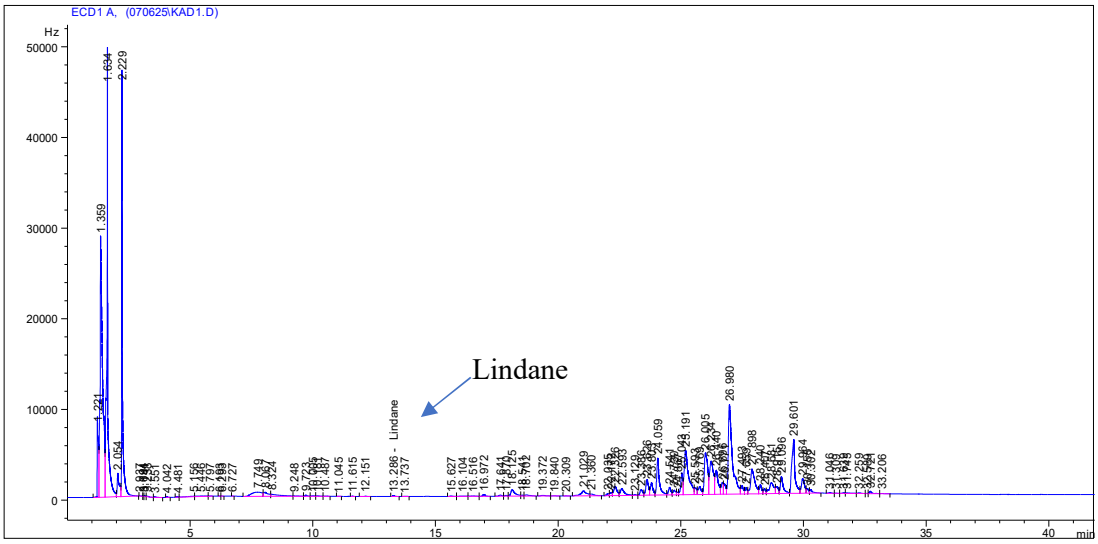

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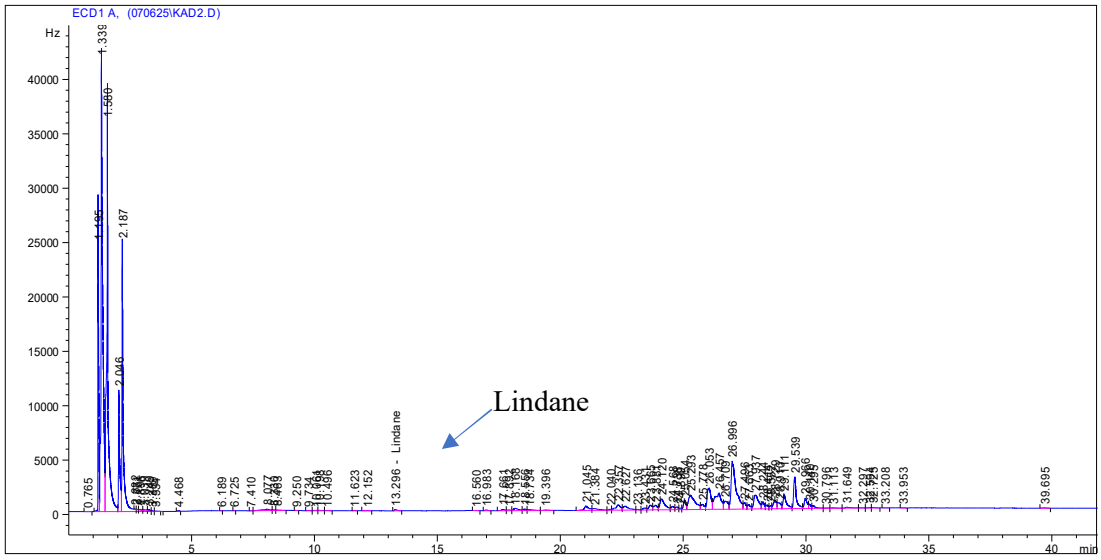

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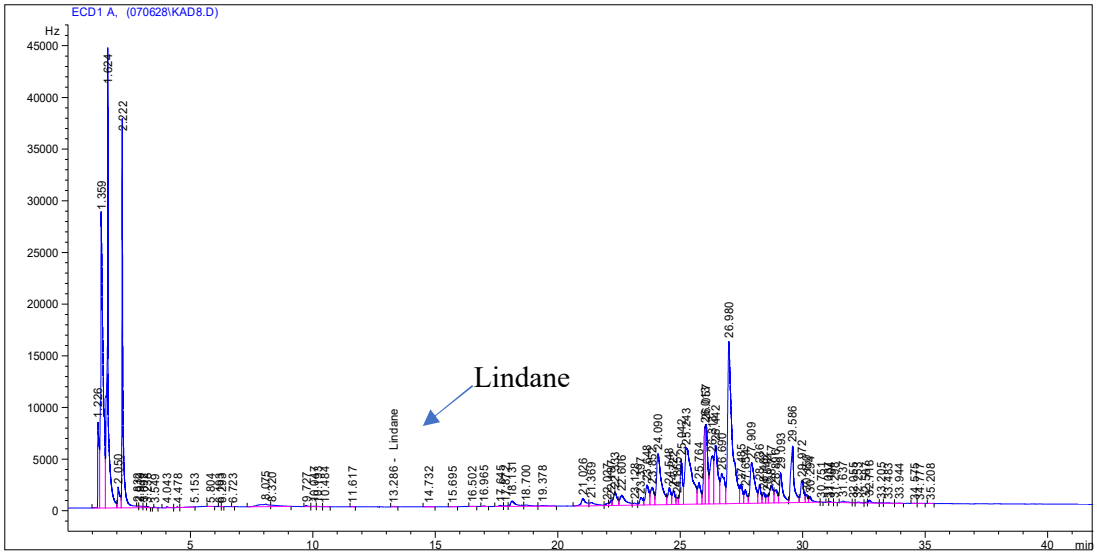

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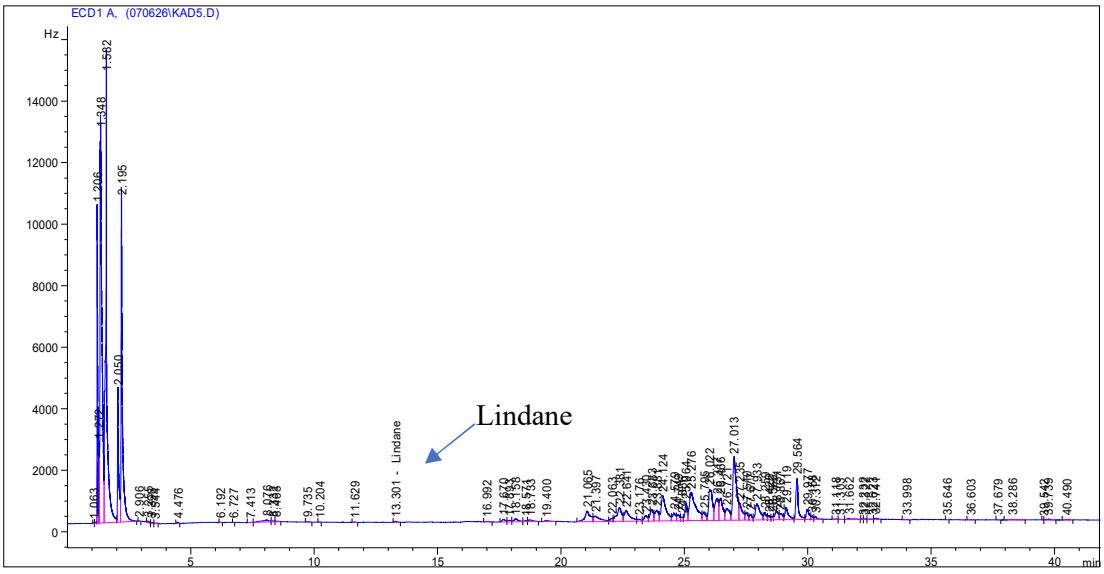

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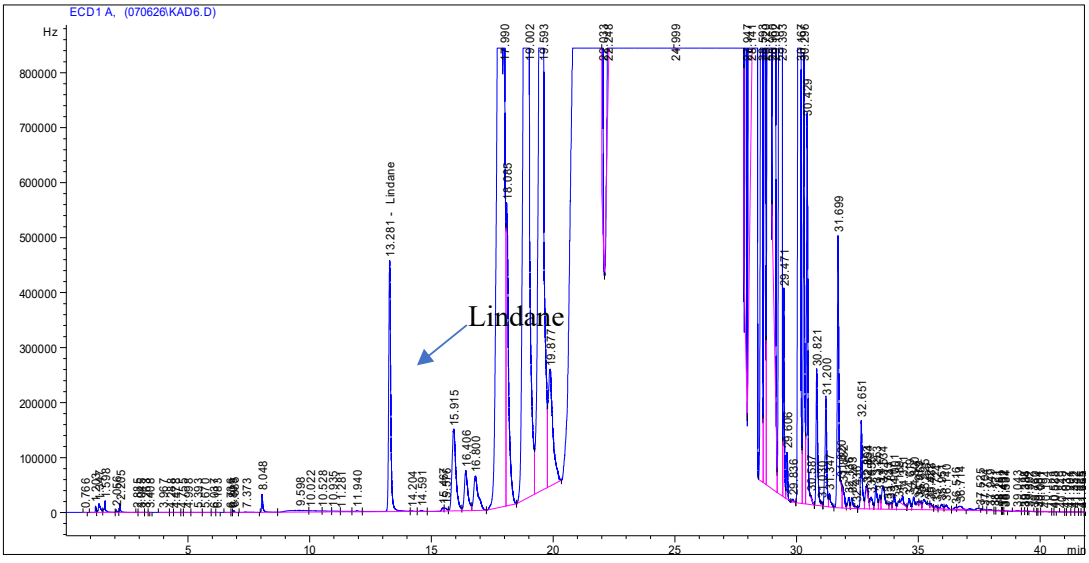

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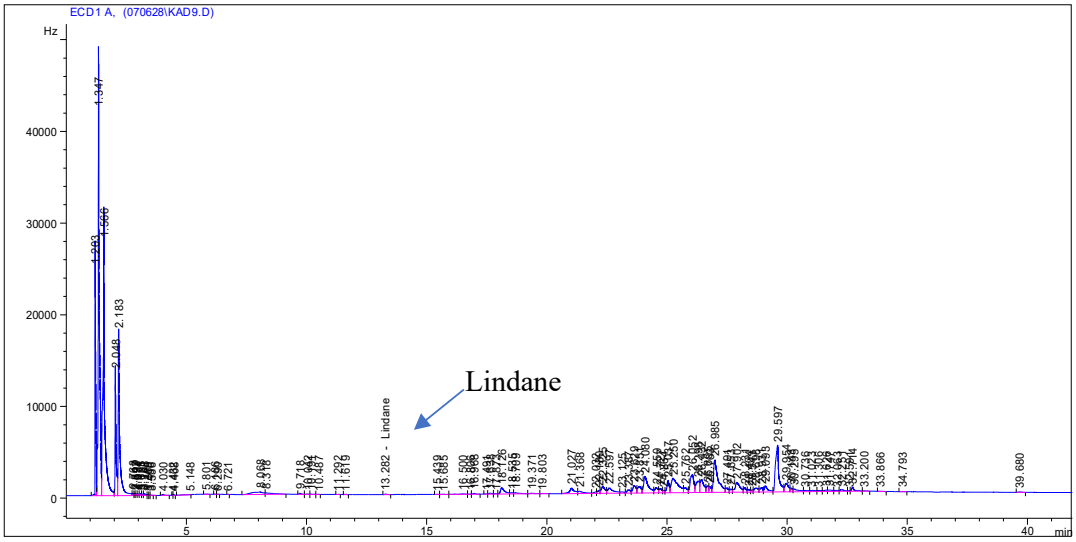


Figure 2. Chromatograms of hexane extract from soil samples after month of incubation. **1**-control soil sample, **2** – soil sample with *Nostoc linckia*, **3-** soil sample with *Nostoc muscorum*, 4 - soil sample with *Nostoc pruniforme*, **5 -** soil sample with *Gloeothece rupestris,* **6 -** soil sample with *Synechococcus cedrorum*, **7 -** soil sample with association of cyanobacteria *N. pruniforme + Gloeothece rupestris*

Table 1. Concentration of hexachlorocyclohexane in the soil samples after a month of incubation

№	Soil sample	Concentration of hexachlorocyclohexane (lindane), ng/g of soil
1	Control sample	2041.56
2	Soil sample with *Nostoc linckia* 4	14.34
3	Soil sample with *Nostoc muscorum* 14	14.36
5	Soil sample with *Nostoc pruniforme* 20	14.21
6	Soil sample with *Gloeothece rupestris* 15	14.20
7	Soil sample with *Synechococcus cedrorum* 12	810.43
8	Soil sample with *N. pruniforme* 20 + *Gloeothece rupestris* 15	14.24

It was previously reported that lindane levels in culture supernatants of *M. aeruginosa* PCC 7806 treated with 7 mg/l lindane were significantly reduced after 15 days of treatment [27]. It is reported that cyanobacteria of the genus *Anabaena, Phormidium, Oscillatoria* destroy various aromatic compounds, *Microcoleus chthonoplastes*, *Phormidium corium, Synechocystis* sp. decompose n-alkanes up to 25% within 7-10 days [29].

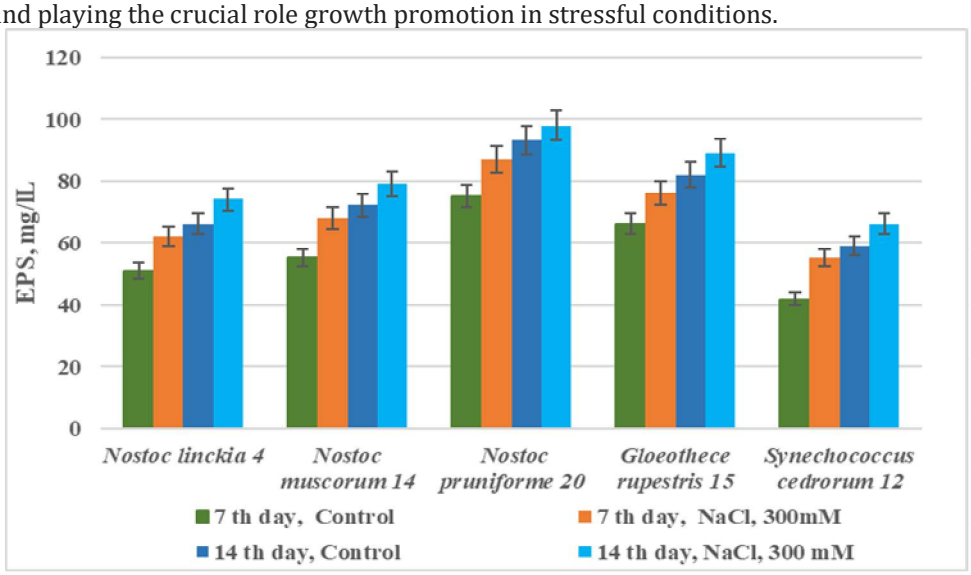
Results of our research allow to make conclusion about high efficiency hexachlorocyclohexane degradation in model conditions in saline soil (4% NaCl) by all investigated cyanobacteria strains and associations. Thus, the received results allow to recommend investigated cyanobacteria and their associations as biological preparations for decontamination of saline soils, strongly polluted by persistent chloroorganic compounds.

It was also revealed that the studied cyanobacterial strains produce exopolysaccharides (EPS) under salt stress conditions. When cyanobacteria *N. linckia* 4, *N. muscorum* 14, *N. pruniforme* 20 and *G. rupestris* 15 were cultivated for 7 days at a sodium chloride concentration of 300 mM, EPS production increased compared to the control by 25.5%; 23.6%; 16% and 15.1%, respectively (Fig. 3A). While, when cultivating cyanobacteria *N. pruniforme* 20 and *G. rupestris* 15 within 14 days under salinity conditions, EPS production increased by 5.4% and 2.1% compared to the control. It should be noted that EPS production increased in *N. linckia* 4, *N. pruniforme* 20 and *G. rupestris* 15 during 14 days of cultivation under conditions of 300 mM salinity in relation to 7 days of cultivation by 45.1%, 30.6% and 34.8%, respectively. One of the most important stress factors is salt stress for microorganisms. Consequently, EPS protect bacterial cells from desiccation, heavy metals, pesticides, or other environmental stresses,

including host immune responses, and also form biofilms, thereby increasing the ability of microorganisms to colonize specific ecological niches [30-32].

It has been established in our study that under salt stress conditions, local strains of cyanobacteria produce the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase. Cultivation of the studied cyanobacterial strains for 14 days showed that the production of ACC deaminase in the cyanobacteria *N. linckia* 4, *N. muscorum* 14, *N. pruniforme* 20, *G. rupestris* 15 and *S. cedrorum* 12 under salt stress conditions (300 mM, NaCl) increased relative to the control by 38.7%, 33.3%, 34.1%, 40.5% and 29.5% after 7 days of cultivation (Fig. 3B). The ACC deaminase is known to play an important role in reducing stress from both biotic and abiotic stressors in plants and thereby enhances their growth under unfavorable environmental conditions [33]. The AcdS gene, encoding ACC deaminase, has been shown to be present in various microorganisms, including bacteria, fungi and endophytes [34]. Plant growth promoting bacteria (PGPB), which have ACC deaminase activity, play an important role in reducing/mitigating the toxic effects of several environmental stressors such as salinity, drought, heavy metals, and organic pollutants [35]. PGPB containing ACC deaminase support plant growth by modulating stress ethylene levels and breaking down its precursor 1-aminocyclopropane-1-carboxylic acid (ACC) into α-ketobutyrate and ammonia. Here we present that cyanobacteria are also capable of production of ACC deaminase and playing the crucial role growth promotion in stressful conditions.

A)



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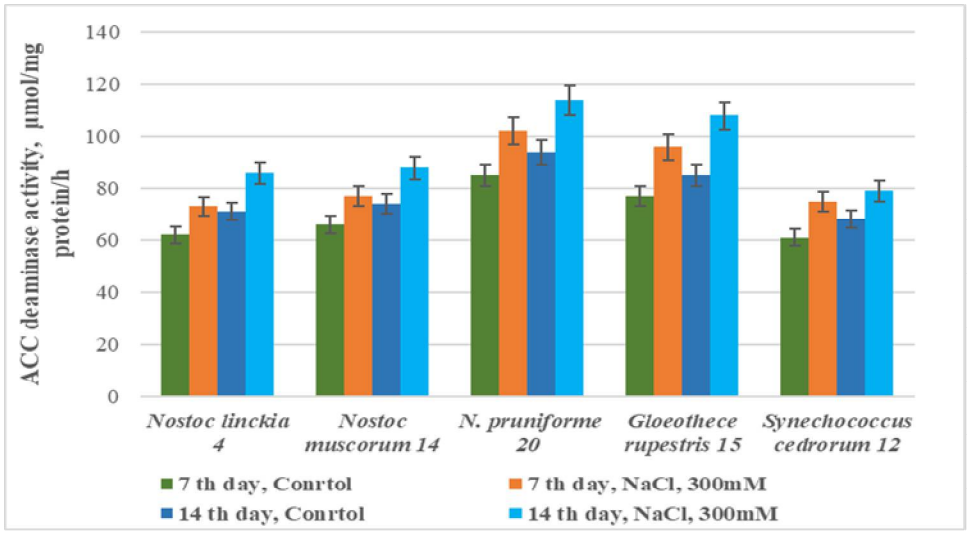

Figure 3. A) EPS content produced by cyanobacteria at 300 mM NaCl stress, B) Quantitative analysis of ACC deaminase enzyme activity of cyanobacteria under salt stress conditions

CONCLUSIONS

Thus, the data obtained give reason to believe that the studied local strains of cyanobacteria *N. linckia* 4, *N. muscorum* 14, *N. pruniforme* 20, *Gl. rupestris* 15 and *S. cedrorum* 12 degrade or transform lindane into

volatile compounds or completely utilize it in conditions of 4% salinity for 1 month in model soil conditions. Also, under salt stress conditions, the studied cyanobacterial strains produce EPS and ACC deaminase. Consequently, EPS and ACC deaminase containing cyanobacteria protect their own cells from desiccation, pesticides, including host immune responses, thereby increasing the survival of cyanobacteria under conditions of salt stress and lindane pollution. Since HCH is almost completely degraded by local strains of cyanobacteria, it is likely that when cyanobacteria are incubated with lindane, enzymes involved in the breaking of pesticides such as polyphenol oxidase, phenol oxidase, catalase, superoxide dismutase, etc. are induced. Cyanobacteria are excellent accumulators or degraders of various environmental pollutants, such as heavy metals, pesticides and oil-containing compounds. In this context, the results obtained in this study allow us to recommend the isolated local strains of cyanobacteria and their associations as the basis of biological products for the purification of saline soils heavily contaminated with persistent organochlorine compounds. Given the limited information on HCH degradation by cyanobacteria, this study provides new knowledge on the remediation of lindane-contaminated soils. The study of key enzymes in the biodegradation pathway of lindane by cyanobacteria requires further in-depth study.

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CONFLICT OF INTEREST
The authors declare no conflict of interest.

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