ISSN: 0975-766X
CODEN: IJPTFI
Available Online through ***Research Article***
www.ijptonline.com

EFFECTS OF CADMIUM AND GLUCOSE ON MICROBIAL COMMUNITIES: REVEALING OF THE LEADING FACTOR

Aliya Ramilevna Gilmullina, Polina Yuryevna Galitskaya, Anatoliy Aleksandrovich Saveliev, Svetlana Yuryevna Selivanovskaya
Department of Applied Ecology, Institute of Environmental Sciences, Kazan Federal University, Kazan, Russian Federation.

Received on 14-08-2016 *Accepted on 20-09-2016*

Abstract

Anthropogenous activity leads to accumulation in the soil of metals which often come to it along with organic matter. In the conditions of a laboratory experiment response of soil microbial community to an individual and simultaneous importation of glucose and cadmium is investigated. Changes of microbial community characterized by the following parameters – the common microbial biomass, respiratory activity and growth characteristics of soil community. It is established that the importation of cadmium and glucose renders multidirectional effects. At simultaneous influence of available organic matter and metal of change of the analysed parameters depend on a ratio and concentration of connections. The cluster analysis of the obtained data demonstrates that at the studied concentration of cadmium and glucose a major factor, in charge of formation of communities, metal whereas organic matter plays the supporting role is.

Keywords: Cadmium, glucose, microbial biomass, respiration, soil communities

Introduction

The soils polluted by metals cause serious concern in many countries. This concern is bound generally to intake of metals in a human body with production which is grown up on the polluted soils [1–3]. Not less important problem is impact of metals on soil communities as the soil provides circulations nutritious [4–7]. Accumulation of metals in the soil happens owing to anthropogenous activity: work of power plants, mining enterprises, means of protection of plants, combustion of naphtha and coal, transport activity. Often metals come to the soil together with organic compounds: at placement because of the industrial organic wastes, rainfall of sewage and their composts, simultaneous application of organic and mineral fertilizers [3, 5, 8–11]. Cadmium, potentially toxiferous element which is widely found in a geosphere in slight quantities (on average 0,2 mg/kg) which content in soils at

anthropogenous influence can reach 500-1000 mg/kg [3, 6] is among the most dangerous metals. Change of physical and chemical, biochemical properties of soils, structural change and activities of community and, finally, efficiency loss can be result of receipt to the soil of metals. In literature the differing data on doses of the metals causing inhibition of functions of microorganisms in the soil and also on their influence in field and laboratory conditions are submitted significantly [3, 10, 12–18].

At assessment of influence of metals most often analyze the soil parameters which are integrally reflecting a condition of microbial communities [5, 19]. An important component of the soil is biomass. However, in spite of the fact that microbial biomass is considered the sensing indicator, many authors note significantly the differing effects rendered by metals on biomass [3, 5]. The cooperative mineralization of organic matter microorganisms is reflected by such parameter as a microbial respiration [3, 10, 16]. There is an opinion that the microbial respiration is more sensitive in relation to metals in comparison with such parameter as microbial biomass [16].

Recently the question and of influence of organic matter on soil processes is actively considered in literature. It is known that the microbial community of the soil functions in the conditions of a limit of organic matters, especially carbon. For the analysis of mechanisms of overcoming by microorganisms of this limitation often use reception in which bring an available source of carbon in the soil. As a model source in the basic glucose is considered, there are publications and about alanine, fructose [20]. It is shown that response of microbial community depends not only on quantity of the brought carbon substratum and its ratio with the content of soil carbon, but more on a ratio with microbial biomass [20, 21]. Thus, in literature the effects caused by receipt to the soil of metals, in particular cadmium, organic matter and glucose as his representative, however, is not enough data on what of factors at their simultaneous influence is leading in formation of microbial community are presented.

The purpose of the real research was identification of the leading factor when forming communities at a simultaneous importation to the soil of metal and organic matter (on the example of cadmium and glucose).

Methods

In work used the soil selected in Alexeyevsky district of the Republic of Tatarstan (Russia) which was characterized by the following parameters: content of the common organic carbon - 6,7%, the common nitrogen – 0,15%, soluble organic carbon – 0,05%, the common microbial biomass – 0,81 mg mc/g and basal respiration – 3,32 mkg of CO2/g*ch. On distribution of sizes of 40,5% - sand, 54,1% - dust, 5,5% - clay.

For each option of processing prepared three independent incubative vessels containing 1 kg of the soil. The soil was

humidified to 60% of water holding capacity. Once a week the soil were mixed and water content was restored by weight. In an experiment analyzed 8 options: s – control initial soil; glu1000 – the soil in which brought 1000 mg/kg of glucose, glu10000 – the soil in which brought 10000 mg/kg of glucose, Cd400 – the soil with the brought cadmium in number of 400 mg/kg, Cd600 - the soil with the brought cadmium in number of 600 mg/kg, Cd600glu10000-the soil with a simultaneous importation of cadmium of 600 mg/kg and glucose of 10000 mg/kg, Cd600glu1000 - the soil with a simultaneous importation of cadmium of 600 mg/kg and glucose of 1000 mg/kg and Cd4001000 - the soil with a simultaneous importation of cadmium of 400 mg/kg and glucose of 1000 mg/kg. Soils incubated within 7 days and selected exemplars for the 1,3,5 and 7 days. From each incubative vessel selected three exemplars, mixed and analyzed soil microbial biomass, basal respiratory activity and growth characteristics of communities.

Basal respiration activity of soil was determined on the basis of CO2 emission from soil, according to ISO 16072 [22]. Soil microbial biomass (Cmic) was determined by fumigation of the samples with C2H5OH-free CHCl3 and extraction with 0.5 M K2SO4. The extracted C content was determined by bichromate oxidation in accordance with ISO 14240-2 [23]. Definition of growth characteristics of soil community was carried out on change of the optical density which is carried out by the automated method by means of the Thermo Scientific Multiskan FS microplate reader device. Beforehand received soil suspension (a ratio 1:10). In the small cavities of the tablet dug out 20 mcl soil suspension and 180 mcl the fluid environment with glucose (g/l): KH2PO4 – 3, MgSO4 · 7H2O – 0,2, NaH2PO4 · 12H2O - 4.5, (NH4) 2SO4 - 1, glucose - 20.

The data from the experiment were statistically processed on a computer using Origin 8.5 (OriginLab, Northampton, USA). The confidence of data generated in the present investigations has been analyzed by standard statistical methods to determine the mean values and standard errors (S.E.). The means were compared using Fisher's Protected Least Significant Difference at $\alpha = 0.05$.The values in figures were expressed as mean ± S.E. of the corresponding replicates.

Results and discussion

Cumulative curves of respiratory activity of the soil, are submitted in fig. 1. Respiratory activity of the control soil fluctuated from 0,02 to 0,04 mg of the soil CO2-C/g a day, and by 7 days of an experiment the cooperative quantity of the allocated carbon dioxide made 0,21 mg of the soil CO2-C/g. At the beginning of the experiment glucose in two concentration – 1000 and 10000 mg/kg was brought in the soil. In case of the former made a ratio 2:1, in the second

is ten times more larger. It is interesting to note that when using as organic fertilizers of a dung or composts about 2-6 mg With on g of the soil [24, 25] are brought in the soil, and the ratio of the brought carbon with carbon of microbial biomass can make about 10 [26].

The importation to the soil of glucose in number of 1000 mg/kg led to increase in respiratory activity, and reliable distinctions with control option observed in 8 clocks of incubation of exemplars. By the end of an experiment the quantity of the allocated CO2 in option with an importation of glucose of 1000 mg/kg exceeded control option by 2,6 times. In glu10000 option in 12 clocks the quantity of the allocated CO2 was higher in experienced option by 8 times, and through 24 – by 25 times. Thus it is possible to assume that activation of soil microorganisms took about 12 hours. It confirms opinion of Blagodatskaya and Kuzyakov [21] that at the soil there is the potentially fissile biomass of microorganisms which quickly (of several minutes till several o'clock) passes into the fissile state. The importation of cadmium led to decrease in respiratory activity, however, this decrease was not the so considerable: by the end of an experiment the quantity of CO2 in Cd400 and Cd600 options appeared in 1,4 and 1,5 times below monitoring. The negative impact of cadmium on respiratory activity was noted also by other authors, and the range of the concentration of cadmium causing negative effect is rather wide [4, 10, 27]. Authors connect distinctions in effects first of all with distinction in the content of organic matter in the soil.

When comparing respiratory activity of exemplars with a simultaneous importation of metal and glucose with values of parameter in soils with cadmium it is possible to see that the importation of glucose restores activity of community, reducing toxic effect of metal. At the same time, than the content of glucose at the identical content of cadmium is higher (Cd600glu10000 and Cd600glu1000 options), that significant increase in a respiration (in 12 and 2,5 times respectively). At an identical dose of glucose, but the differing amount of metal of distinctions in respiratory activity it is not revealed. It should be noted that the activation by glucose of microbial community which is reflected in increase in respiratory activity in exemplars happens to cadmium later (since the 48th hour of an experiment), than at an importation only of glucose.

Results of definition of microbial biomass in soil exemplars are presented in fig. 2. Content of microbial biomass in an initial exemplar made 0,2 mg/g. Within 7 days the level of biomass was subject to fluctuations and at the end of an experiment made 0,13 mg/g. The glucose importation in number of 1000 and 10000 mg/kg led to slight increase in biomass which at the beginning of the experiment made 110 and 131% of biomass of control option. According to data of literature, a glucose importation in quantity larger, than carbon of biomass can be followed by increase in

biomass as this carbon is used not only in catabolic, but also anabolic processes [21]. In our case reliable increase in biomass (for 30%) was observed only in case of a glucose importation in number of 10000 mg/kg when a ratio Sglyu:smikr makes 20:1. Decrease in microbial biomass lower than the level of control option is revealed in exemplars of soils with an importation individually of cadmium. The greatest distinctions are revealed at the beginning of the experiment: biomass in soils of Cd400 and Cd600 options made 60 and 55% of control option. Such effect is a consequence of toxic effect of cadmium and rather explicitly is described in literature [28, 29].

The simultaneous importation to the soil of cadmium and glucose resulted in the following effects. At the beginning of the experiment the level of microbial biomass in exemplars of Cd600glu1000 and Cd600glu10000 is significantly lower than monitoring (51 and 56%), and the content of glucose practically does not influence its values. At the same time in Cd400glu1000 exemplar biomass level practically does not differ from monitoring level. By the end of incubation the toxic effect of cadmium is leveled by effect of glucose and the level of microbial biomass is restored to the level of control option. Body height of microbial culture depends on the initial maintenance of microorganisms therefore characteristics of curves of body height of soil microbial communities can reflect quantity and a condition of microorganisms in exemplars. Apparently from data of the figure 3, the community of the control soil showed almost identical body height throughout all experiment. Curves of body height of microbial communities of exemplars of glu10000, were characterized by similar duration a log phase except for the test which is selected for the 7th days of incubation of an exemplar: in this case a log phase it was reduced till 12 o'clock and in 3 days of body height of microorganisms, the optical density was 1,2-3,2 times higher in comparison with control option. At communities of exemplars glu1000 the smaller optical density, in comparison with glu10000 exemplars is established, however, it was significantly higher that in option s. Thus, glucose renders the stimulating effect, increasing number the saprotrophic of organisms in the soil at the expense of an importation of a padding nutritious substratum. In exemplars of Cd400 which are selected for the first days of an experiment a total optical density of culture was 2,7 times less in comparison with monitoring. In the tests which are selected at later stages of distinction in an optical density become not reliable that, most likely, is bound to adaptation of microbial community to the brought metal. The larger effect is revealed at an importation to the soil of cadmium in number of 600 mg/kg. So, microbial communities of the exemplars which are selected in the first and second day showed lack of body height and only since fifth days there is slightly a restitution of community. The simultaneous importation in an exemplar of cadmium and glucose in different ratios results in the following results. Glucose in number of 10000 mg/kg reduces

negative effect of metal in exemplars with cadmium high content only since 5 days, and for the 7th days the similar effect is noted also in exemplars with glucose of 1000 mg/kg.

Thus, for each exemplar processed in various ways the data allowing to characterize integrally changes in microbial communities at a given time were obtained. To define the most significant factor in formation of microbial communities, the cluster analysis was carried out. As objects for the analysis used the tests which are selected four times (for 1, 3, 5 and 7 days) in the course of incubation of soils (8 options). Thus, 32 objects participated in the cluster analysis. Each of objects was characterized by three indexes: respiratory activity, the common microbial biomass and ability to grow on the Wednesday with glucose. As a result of the hierarchical cluster analysis which is carried out by Ward's method with use of Euclidean distance, all objects will obviously unite in three clusters which we will number from below up (fig. 4.). The most removed from others there was the first cluster which part all exemplars (which are selected four times throughout an experiment) with the metals brought individually (were at both concentration), and also all exemplars containing at the same time cadmium in high to (600 mg/kg) and glucose in low concentration. Thus, it is possible to assume that at the studied concentration of cadmium and glucose the first factor, in charge of formation of communities, metal is. Cd600glu10000 exemplar which is selected at the initial moment of an experiment that demonstrates proximity of its characteristics to those of the exemplars which are strongly polluted by cadmium entered the same cluster. Other exemplars were in turn united in two clusters (second and third). The second cluster included all control specimens in which glucose in number of 1000 mg/kg (except for an exemplar of glu1000 which is selected at the end of an experiment which entered the third cluster) and all exemplars with the low content of cadmium (400 mg/kg) and glucose (1000 mg/kg) was brought. Join of the specified exemplars in one cluster with control testifies to similarity of their characteristics. All exemplars in which brought glucose in a great many (10000 mg/kg), and also three exemplars which are selected for the 3,5 and 7 days of an experiment with the high content of cadmium, but also high content of glucose were a part of the third cluster. Thus, distribution of exemplars on clusters demonstrates that, most likely, the second factor defining formation of microbial communities is availability of glucose and its concentration.

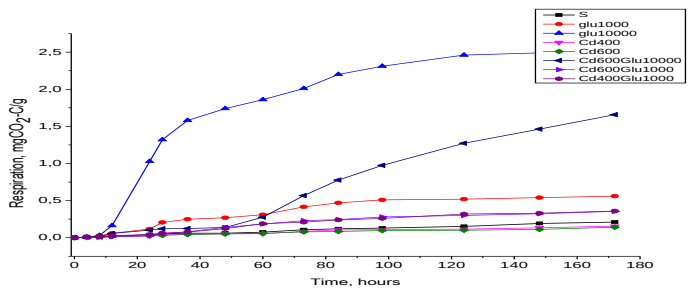


Fig. 1: Cumulative curves of the respiratory activity of the soil amended with glucose and cadmium. s - control the initial soil; glu1000 -pochva, which made 1000 mg / kg of glucose, glu10000 -pochva, which contributed 10,000 mg / kg of glucose, CD400 - Adding to the soil with cadmium in an amount of 400 mg / kg, Cd600 - Adding to the soil with cadmium in an amount of 600 mg / kg, Cd600glu10000- soil with simultaneous application of cadmium 600 mg / kg of glucose and 10 000 mg / kg, Cd600glu1000 - soil with simultaneous application of cadmium 600 mg / kg and 1000 mg glucose / kg Cd4001000 - soil with simultaneous application of cadmium 400 mg / kg glucose 1000 mg / kg.

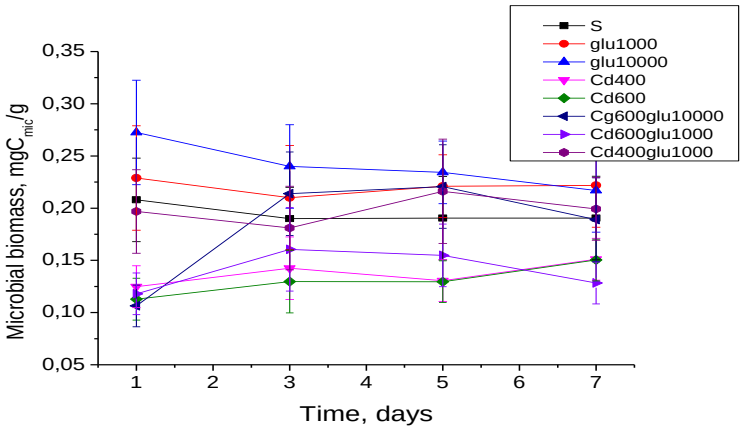
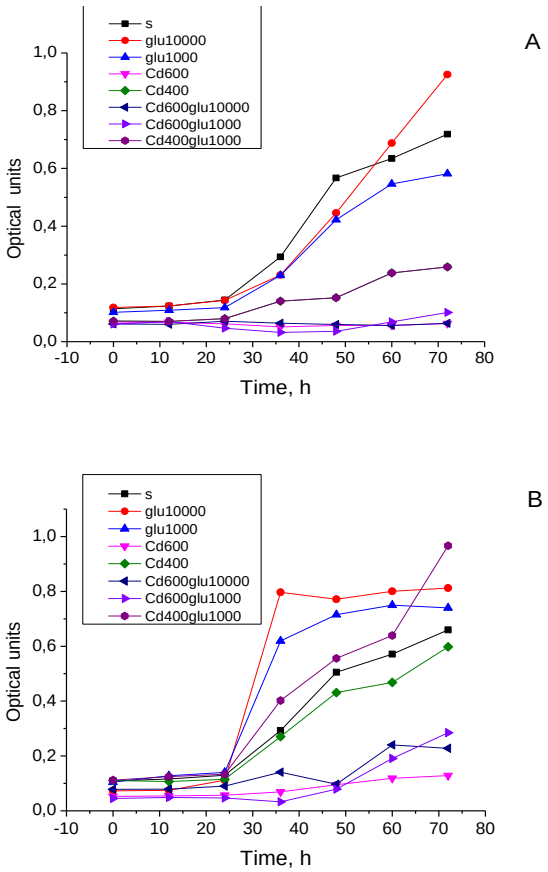


Fig- 2: Changes of microbial biomass soil samples amended with glucose and cadmium. Symbols as in Figure.1.



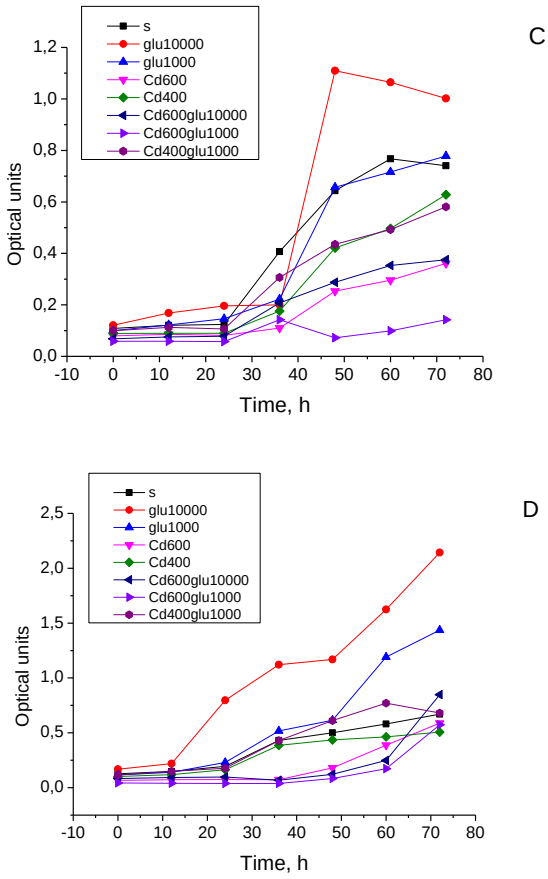


Fig.3: Growth curves of communities of microorganisms isolated from soil samples, and amended with glucose cadmium 1 (A), 3 (B), 5 (C) and 7 (D) per day. Symbols as in Figure. 1.

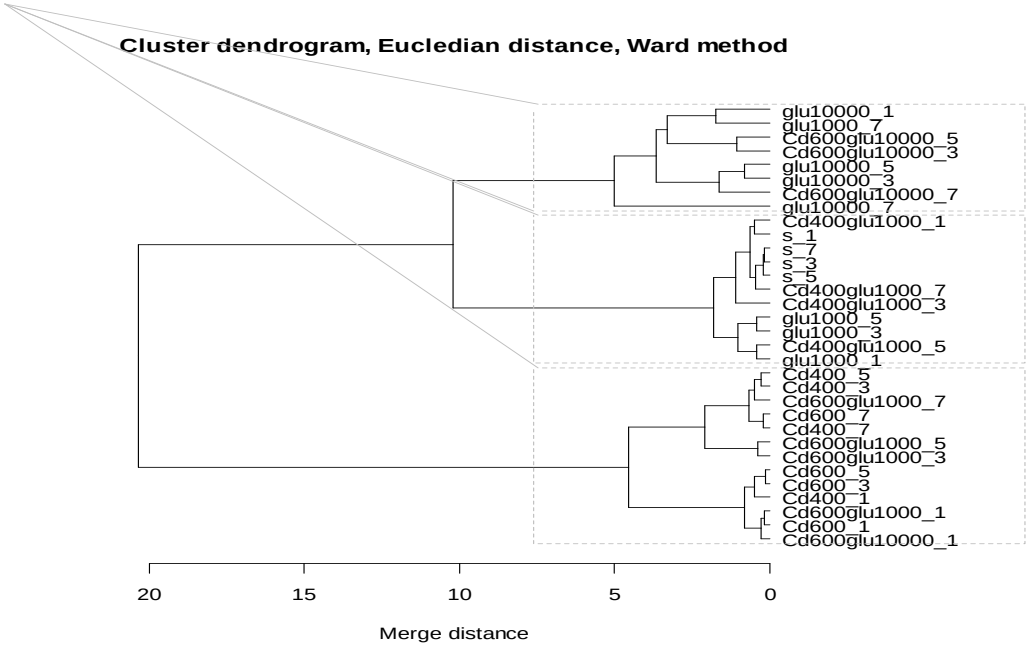


Fig. 4. Dendrogramma of the klastarny analysis.

Conclusion

On the basis of the analysis of change of the integral indexes characterizing a condition of microbial communities it is possible to conclude that the importation of cadmium and glucose renders the multidirectional effects depending on

concentration of the brought connections. Individually brought cadmium (400 and 600 mg/kg) inhibits respiratory activity, reduces microbial biomass and body height of microbial community of the soil, at the same time cadmium renders doze-dependant effect which in case of two last parameters is leveled by the end of an experiment. Glucose renders the stimulating effect on all analysed parameters, and the most expressed effect was observed at a glucose ratio from microbial biomass 20:1. The importation of an available substratum leads at the same time to increase in respiratory activity, microbial biomass and body height of community. Decrease in quantity of an available substratum at the expense of the fissile mineralization of glucose at the initial stage is resulted decrease in microbial biomass and number by the saprotrophic of organisms which is reflected in change of curves of body height of community in a rich medium.

At simultaneous influence of available organic matter and metal of change of the analysed parameters depend on a ratio and concentration of the brought connections. The cluster analysis of the obtained data demonstrates that at the studied concentration of cadmium and glucose a major factor, in charge of formation of communities, metal whereas organic matter plays the supporting role is.

Acknowledgments

Work is performed with assistance of a grant of the Russian Federal Property Fund No. 15-04-04520.

The work is performed according to the Russian Government Program of Competitive Growth of Kazan Federal University.

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