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Impact of Gold and Silver Nanoparticles on Terrestrial Flora and Microorganisms

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***Abstract*—**Despite the rapid nanotechnology progress and recognition, its potential impact in ecosystems and health of humans is still not fully known. In this paper, the study of ecotoxicological dangers of nanomaterials is presented. By chemical reduction method, silver (AgNPs) and gold (AuNPs) nanoparticles were synthesized, characterized and used in experiments to examine their impact on microorganisms (*Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*) and terrestrial flora (*Phaseolus vulgaris* and *Lepidium sativum*). The results collected during experiments with terrestrial flora show tendentious growth stimulations caused by gold nanoparticles. In contrast to these results, silver nanoparticle solutions inhibited growth of beans and garden cress, compared to control samples. The results obtained from experiments with microorganisms show similarities with ones collected from experiments with terrestrial plants. Samples treated with AuNPs of size 13 nm showed stimulation in the growth of the colonies compared with 3,5 nm size nanoparticles.

***Keywords*—**Ecosystems, ecotoxicology, nanomaterials, nanoparticles.

I. Introduction

LATELY, nanomaterials, which represent a wide specter of materials, are being used in various fields, from engineering to biomedicine. Undoubtedly, distinctive properties of nanomaterials had solved many engineering challenges. Unusual properties of nanoparticles mainly depend on a high aspect ratio of its dimensions. As a result, nanoparticles have incredible properties that are different from the bulk material. For instance, in nanoscale changes optical properties of the material, also melting temperature is lower, and metallic nanoparticles have a high Surface Plasmon resonance [1]. Gold and silver nanoparticles are one of the most widely used particles. For example, gold nanoparticles had been used for the centuries by the artists because of their bright colors in stained glass. Nowadays, gold nanoparticles are used in electronic chips, photodynamic therapy, sensors and importantly, for the drug targeting because of their property to attach themselves to biomolecules [2]. Meanwhile, the substantial antibacterial properties of silver nanoparticles are being broadly used in bandages, biological and chemical sensors, photo-electrodes and clothing. Obviously, nanomaterials and, specifically, nanoparticles (NPs) are becoming a part of our daily used products and, consequently, the interaction of the nanoparticles with humans and environment is increasing. Nanoparticles can enter ecosystems in various ways: During synthesis, transportation, product lifetime or waste [3]. When in the environment, nanoparticles can be affected and affect the surroundings [3]. As the nanoparticles contact with the environment is increasing, appears the need to know how the use of nanomaterials will influence various organisms in the environment. Despite the high possibility of nanoparticles getting into the environment, there is little known how it would act in the ecosystems. To this day conducted nano-ecotoxicological studies still cannot declare the toxicity or acquit nanoparticles from being possibly toxic for the environment. More studies carried out on prolonged effects of nanoparticles might help to find out if nanoparticles are toxic or not.

In this paper, report of gold and silver nanoparticles impact on microorganisms and terrestrial flora is presented. The impact of nanoparticles on microorganisms and terrestrial flora was investigated by introducing nanoparticles into a culture media or compost. It was found that nanoparticles effects are tendentious. Gold nanoparticles stimulated the growth of microorganisms and terrestrial flora. Silver nanoparticles, on the other hand, inhibited growth of test subjects.

II. Materials and Methods

A. Culture Media

Trypticase soy agar (TSA) culture media was previously prepared in the laboratory (YR lab, LMNSC) for microorganisms studies.

B. Strains of Microorganisms

Gram negative bacteria *Escherichia coli* (ATCC 25922 strain), Gram positive bacteria *Staphylococcus aureus* (ST 1 strain) and micro fungi *Candida albicans* (ATCC 10231 strain) were previously isolated in laboratory (LMNSC, YR lab) and provided by P. Kavaliauskas from Vilnius University. The strains were subcultured and used throughout the study.

C. Terrestrial Flora

The seeds of common white bean *Phaseolus vulgaris* and garden cress *Lepidium sativum* were purchased from UAB agro firm "Sėklos", Lithuania.

D. Preparation of Nanoparticles

a) Synthesis of Gold Nanoparticles

Gold nanoparticles (AuNPs) were synthesized by chemical

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citrate reduction method first brought by J. Turkevich and refined by G. Frens [4], [5].

During the first step of synthesis, two primary solutions were prepared (solution A and B). Solution A was made by mixing 79 ml deionized (DI) H_2O and 1 ml 1% $HAuCl_4$. Solution B was made by mixing 4 ml 1% trisodium citrate ($Na_3C_6H_5O_7$) and 16 ml DI H_2O and certain amount of tannin acid ($C_{76}H_{52}O_{45}$), depending on particles size [6]. The particles size is smaller – the bigger amount of tannin acid is required. When synthesis of particles, which are smaller than 5 nm, is being performed, the amount of sodium carbonate (Na_2CO_3) must be added for keeping of constant pH level.

A and B solutions were heated and mixed up. The color change of the solution (from transparent to ruby-red) is indicating that the increase of temperature is required. The solution then is heated to +95°C.

Three different size of gold nanoparticles (3,5; 6 and 13 nm in diameter) were synthesized and used in experiments.

b) Synthesis of Silver Nanoparticles

Chemical reduction method [7] was used for preparation of colloidal silver nanoparticles. The DI water was used for preparation of all reacting materials. For typical synthesis, 50 ml of 0,001 M silver nitrate ($AgNO_3$) is heated to boil. To that solution 5 ml of 1% trisodium citrate ($C_6H_5Na_3O_7$ x $2H_2O$) is added drop by drop. During this process, vigorous mixing and heating is required until change of color is evident (from transparent to pale yellow). Then the heating is stopped and the solution is left mixing until cooled to room temperature. Synthesized nanoparticles were

E. Characterization Methods of Nanoparticles

Ultraviolet-Visible spectroscopy (UV/Vis) specters were used to characterize both types of nanoparticles. The UV/Vis absorption of gold nanoparticles have peak on 522 nm indicating all three types of synthesized gold nanoparticles. Meanwhile, UV/Vis spectra of silver nanoparticles have a peak on 400 nm [7].

F. Growth Experiment of the Microorganisms

The experiments to study the effects of nanoparticles on the microorganisms were carried out with three different species (*E. coli*, *S. aureus* and *C. albicans*). Cultures were incubated in saline and solutions of nanoparticles for 1h in 37 °C temperature until turbidity of the culture reached 0,5 McFarland. Cultures were inoculated on Trypticase soy agar and incubated for 24h in 37 °C temperature. After the incubation the Colony Forming Units (CFU) were counted in each plate.

Nanoparticles used in the experiment were diluted in range 1:2 and 1:4 with saline.

G. Growth Experiment of the Terrestrial Flora

Impact of nanoparticles was studied on two species of terrestrial flora: *Phaseolus vulgaris* and *Lepidium sativum.* The effects of gold and silver nanoparticles were examined by watering test subjects with colloidal solutions of nanoparticles. Control groups were watered with water.

For *P. vulgaris* experiment 24 seeds were selected and planted in compost, two in each pot. Beans were watered daily, with 10 ml of three different size solutions of diluted gold nanoparticles.

During the experiment with *L. sativum,* test subjects were watered daily. For the experiment, 120 seeds were selected for each sample. Colloidal solutions of nanoparticles were used diluted in range 1:5 and test subjects were watered with 10 ml. Gold (Ø6 nm) and silver nanoparticles were used for experiment. These plants were treated daily with colloidal solutions of nanoparticles and lightning.

Results were collected by measuring steam of a plant shoot.

III. Results and Discussion

A. Characterization of Gold Nanoparticles

Gold nanoparticles were successfully synthesized using presented method; the rich red color of the colloid indicates the formation of gold nanoparticles.

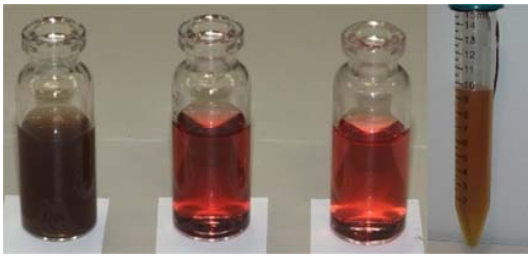

Fig. 1 Synthesized gold nanoparticles. Respectively from left to right: Ø3.5nm, Ø6nm, Ø13nm size nanoparticles and silver nanoparticles

The UV/Vis absorbance spectrum of three different size gold nanoparticles is shown in Fig. 2. Main peaks from 520 to 530 nm indicate strong surface Plasmon resonance.

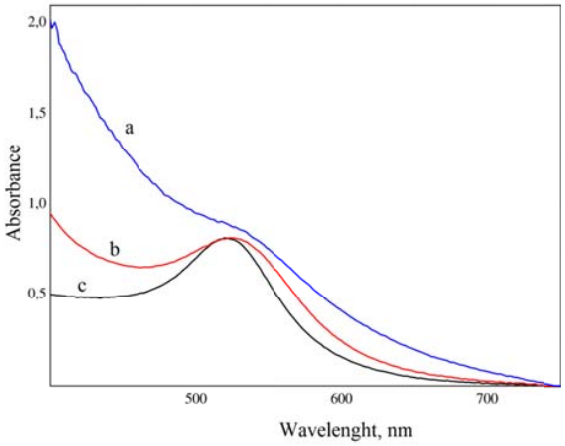

Fig. 2 UV/Vis spectra of gold nanoparticles: (a) Ø3.5nm AuNPs, (b) Ø6nm AuNPS and (c) 13nm AuNPs

The Plasmon absorption depends on shape and size of nanoparticles. There is a smooth shoulder near 460 nm and a shift in the absorption peak to 530 nm.

B. Characterization of Silver Nanoparticles

Synthesis of silver nanoparticles was successful as the change of color was significant (Fig. 1). The solution color change from transparent to brownish yellow proves the formation of silver nanoparticles.

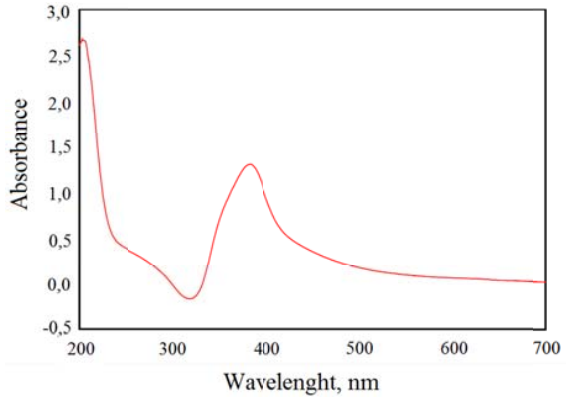

Fig. 3 Absorption spectra of synthesized AgNPs

The absorption spectrum of colloidal solution of silver nanoparticles is shown in Fig. 3. Spectrum shows higher peak at 400 nm wavelength indicating Plasmon resonance absorption. When the nanoparticles is bigger spectrum would shift more closely to 500 nm wavelength.

C. Growth Experiment of the Microorganisms

a) Effects of Gold Nanoparticles to *Candida albicans*

Candida albicans specie was used to study gold nanoparticles effects on micro fungi. After 24h growth incubation *colony forming units* (CFU) were counted in the sample plates.

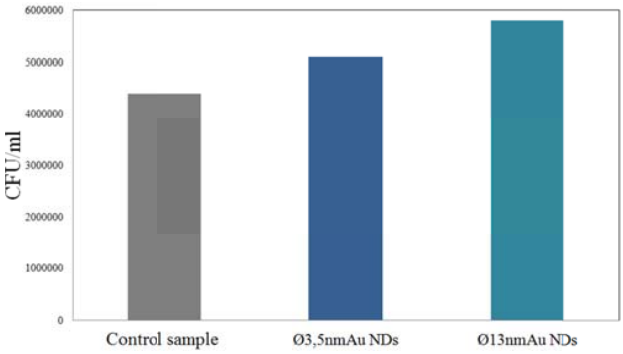

Fig. 4 Diagram showing impact of gold nanoparticles on micro fungi *C. albicans* (CFU) after 24h bacterial growth

The results of experiments of gold nanoparticles effects for the culture growth show a growth stimulation that depends on the size of the nanoparticles. The hugest growth stimulation is noticeable in the sample treated with 13 nm diameter size gold nanoparticles. The untreated micro fungi reached 4,392 x 10^6 CFU per ml compared to 5,1 x 10^6 CFU/ml (3,5 nm AuNPs) and 5,808 x 10^6 CFU/ml (13 nm AuNPs).

Gold ions are known for being used in fertilization products for their properties to stimulate the growth [8]. Nanoparticles are more reactive than bulk material or ions because of bigger ratio of volume and surface area. The presence of nanoparticles probably influenced the fungi cells division by attaching themselves to biomolecules [2]. It could be verified by comparing bacterial growth treated with gold ions and gold nanoparticles of various sizes.

b) Effects of Gold and Silver Nanoparticles on *E. coli*

After 24h culture forming units of *Escherichia coli*, treated with nanoparticles bacterial growth, were counted. The results presented in Fig. 5 shows silver and gold (different concentration and size) impact on *E. coli* bacterial growth.

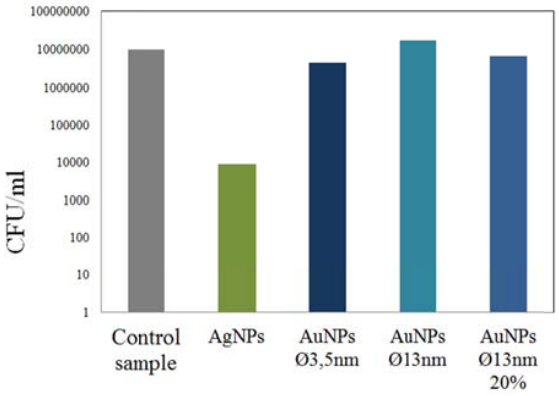

Fig. 5 Comparison of CFU count of *E. coli* (control sample) and under the treatment of silver and gold nanoparticles (different concentrations) after 24h bacterial growth

The obtained results verify antibacterial properties for the Gram-negative bacteria. Compared with control sample, which reached 9,8 x 10^6 CFU/ml, sample influenced with silver nanoparticles reached only 9 x 10^3 CFU/ml. It is possible that not the silver nanoparticles itself are toxic for the growth of the bacteria, but the presence of not reacted silver ions in the solution is affecting the bacterial growth, because only the enormous concentration of silver nanoparticles in the sample might influence the bacterial growth [9]. In that case, the synthesis method of silver nanoparticles should be revised and experiments using different synthesis method should be carried out. On the other hand, as silver nanoparticles are metallic particles there might appear a formation of reactive oxygen species (ROS) and even lipid peroxidation which would lead to growth inhibition [10]. This may result in damage to proteins, oxidative stress, damage to membranes and DNA. This could be considered as mechanisms of nanotoxicity. The oxidation of already synthesized nanoparticles needs research as well as nanotoxicity and possible DNA affections by nanoparticles.

It can be stated, that 13 nm size in diameter gold nanoparticles stimulate the bacterial growth of *E. coli*. This is a similar process as one studied during the experiment with *C. albicans*. Consequently, it was assumed that these processes might be caused by the same effects as in previous

experiment (*C. albicans* experiment). Nevertheless, samples treated with 3.5 nm size gold nanoparticles show the inhibition of bacterial growth. Large surface area of nanoparticles implies that there is more area to come in contact with the cells of bacteria [11]-[14]. The smaller nanoparticles produce electronic effects when in contact with bacteria. These effects increase the reactivity of nanoparticles which could lead to higher antibacterial activity than compared with bigger particles. The study of nanoparticles with diameter bigger than 20 nm could verify it. Different concentration (20% instead of 50%) of 13 nm size gold nanoparticles showed lower bacterial growth.

c) Effects of Gold and Silver Nanoparticles on *S. aureus*

The study results of nanoparticles impact on *Staphylococcus aureus* is shown in Fig. 6. The bacteria were incubated for 24h before counting the CFU.

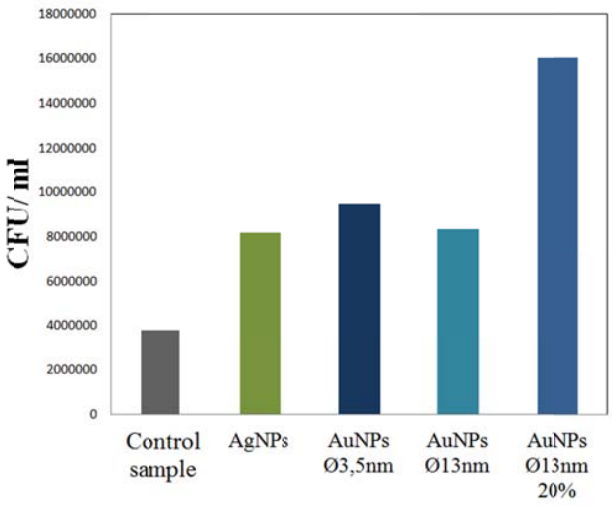

Fig. 6 Comparison of CFU count of *S. aureus* (control sample) and under the influence of silver and gold nanoparticles (different concentrations) after 24h bacterial growth

Different from the experiment with *E. coli*, silver nanoparticles showed no antibacterial effects on Gram-positive *S. aureus*. The reason behind that might be difference between Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria. The different bacteria cell walls will differently react with all types of nanoparticles.

From the results, it can be seen that all samples treated with silver and gold nanoparticles stimulated the bacterial growth of *S. aureus* compared to its control sample. The control sample during this experiment reached only 3,812 x 10^6 CFU per ml, when the in sample, which was treated with 20% solution of 13 nm size AuNPs bacterial growth was 1,6056 x 10^7 CFU/ml. This enormous, compared to control sample, bacterial growth is similar process to the one registered in the experiment with *C. albicans*.

D.Growth of Terrestrial Flora Affected with Nanoparticles

During the experiment obtained results of how different size gold nanoparticles influence growth processes of *Phaseolus vulgaris* are shown in Fig. 7.

Examined common beans (*P. vulgaris*) were watered with AuNPs and control solutions. Beans, which were treated with 13 nm size gold nanoparticles, sprouted first. After other 24h sprouted seeds affected with 6 nm size gold nanoparticles. The samples treated with 3,5 nm size AuNPs sprouted the last. During the experiment, the maximum growth speed was reached using 13 nm size NPs. The speed was about 3 cm per day. The two factors can be indicated for the consideration of further research: the temperature control and selection of wild species or weeds. The possible danger of gold nanoparticles getting into the environment would be affecting the cultivated fields. The leak of gold nanoparticles through the waste or product using could affect the crops, or considering that weeds absorb minerals from the soil faster, it could lead to overgrowth of cultivated fields.

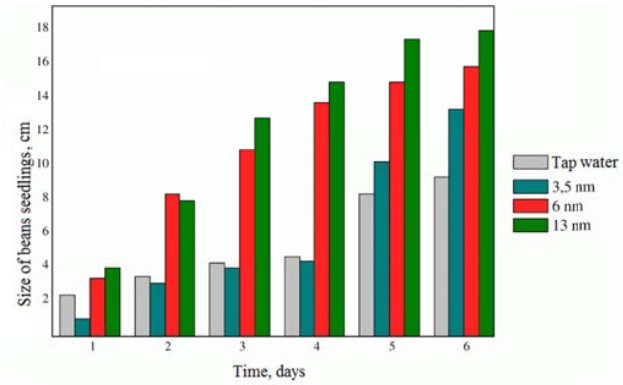

Fig. 7 Growth diagram of *P. vulgaris* treated with gold nanoparticles during first week of the experiment

It is known, that gold ions stimulate the cell division, plant enzymes and stimulates the growth of plants [1]. As the nanoparticles are more reactive than bulk material or even metal ions, it might be the reason of fast growth of the common bean. Other possibilities might be genetic modification or chemical stress which would cause the fastened growth of the test subjects. It could be verified by changing the experiment conditions and growing few generations of affected plants and using gold ions as a control group.

After that, impact of nanoparticles on the growth processes of *Lepidium sativum* was studied in the sequence of the experiments. Firstly, effects of different size of gold nanoparticles were studied. The results were similar to the ones received from the experiment with *P. vulgaris*. The results showed the dependence of the growth on the size of nanoparticles. If particles were bigger, the plant grew bigger as well.

Later, it was decided to conduct experiments with 6 nm size gold nanoparticles and to compare it with effects of silver

nanoparticles. The results of this experiment are shown in Fig. 8.

During this experiment, it was observed, that plants treated with silver nanoparticles were weaker and smaller in size. For comparison, samples treated with gold nanoparticles were higher and healthy looking. All samples ripened the seeds. Only 5% of test subjects influenced with silver nanoparticles ripened the seeds, 8% of control samples ripened the seeds and 50% of test subjects treated with gold nanoparticles ripened seeds (Fig. 9).

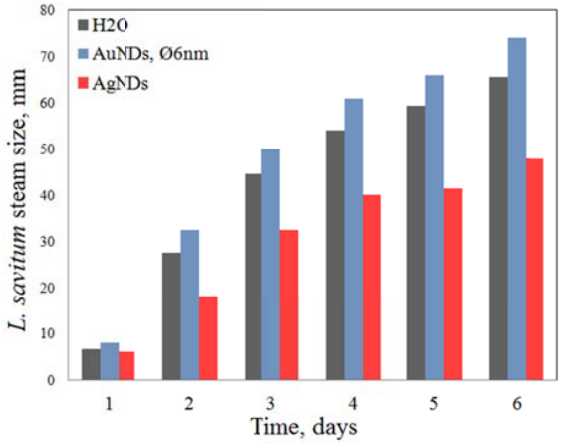

Fig. 8 The comparison of plants treated with different nanoparticles and control samples

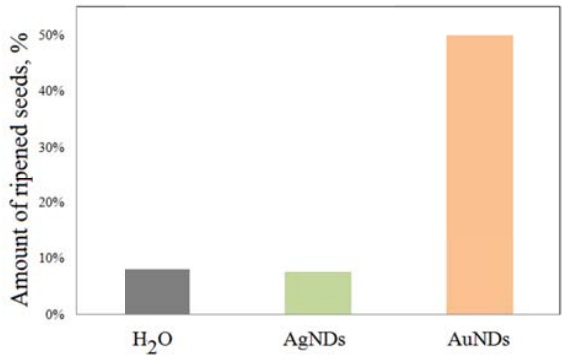

Fig. 9 The comparison of amount of ripened seeds of samples during the experiment

The processes observed during this experiment were similar to the previous experiment; consequently, the reasons behind these processes might be similar to the previous experiment with *P. vulgaris*. Also, it is possible, that used gold nanoparticles are small enough to get into the cell and its nucleus and cause the polyploidization. It would result in similar symptoms as ones observed during the experiment. Although, plant cells are surrounded by a biopolymeric cell wall, which is selective and that makes it hard for nanoparticles enter the cell without damaging the wall. Further research is required.

In terms of studies with silver nanoparticles, the growth inhibition of test subjects was observed. Results collected from stages of germination and early growth might be caused by toxic effects of Ag ions presence in the solution or the toxicological effects of silver nanoparticles. Moreover, it is believed, that nanoparticles that get into the environment by leakage might act as a heavy metal and poison the plant in that way [15].

IV. Conclusions

Nanoparticles and their properties can be beneficial to the humanity and help people to solve serious engineering and health problems. Nevertheless, the effects to other organisms have to be studied in order to know how nanoparticles would act when introduced to environment.

In this presented research, it was observed that the biggest synthesized gold nanoparticles (13nm in diameter) stimulated the growth of microorganisms and terrestrial flora most significantly. On the other hand, effects of silver nanoparticles were quite opposite to ones caused by gold nanoparticles. Silver nanoparticles inhibited growth of microorganisms and terrestrial flora, both types of test subjects.

The results suggest that if both types of studied artificially synthesized nanoparticles would get into the environment it would have effect on plants community, microorganisms, and microflora.

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