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ORIGINAL ARTICLE

Effects of the biogenic gold nanoparticles on microbial community structure and activities

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Abstract This study reports the terrestrial ecotoxicity of the spherical gold nanoparticles with size of 14±3 nm and the negative surface charge of −33±3 mV synthesized by the biomass of *Streptomyces rimosus*. The impact of these gold nanoparticles on the size and structure of microbial communities in the garden soil was analyzed by the enumeration of culturable soil microorganisms and community level physiological profiles (CLPP). Treatment effects on soil enzymatic activities for β-glucosidase, urease, alkaline phosphatase, and dehydrogenase were followed. Toxicity towards soil microorganisms involved in carbon and nitrogen transformation was determined. The data obtained in this study demonstrate that the biogenic gold nanoparticles up to the concentration of 33 mg kg^{-1} do not affect the soil processes and can be classified as "not harmful."

Keywords Gold nanoparticles · Terrestrial ecotoxicity · Garden soil · Microbial community

Introduction

Gold nanoparticles are structures with at least two dimensions between 1 and 100 nm consisting of several tens or hundreds of atoms and may have a variety of sizes and morphologies (amorphous, crystalline, needles, etc.). These particles are creating a new category of materials, which is different either from conventional bulk metal or from atoms and have received great interest due to their attractive electronic, optical, thermal, and catalytic properties. The use of gold nanoparticles in analytical chemistry, medicine, material science, the construction industry, electronic fabrication, etc., has continuously increased in recent years (Sperling et al. 2008; Sadowski and Maliszewska 2011). This increased usage means that an increasing number of nanoparticles will be released to the environment through a production process or after their use.

Metallic nanoparticles are often designed to be extremely reactive, and they have characteristics that may be harmful to different life forms including microorganisms, plants, and animals. They raise serious environmental concerns because of their unique dissolution properties and electronic charges, in addition to their small sizes and large surface-to-mass ratio (Hristozov and Malsch 2009). Thus, the unique properties that make gold nanoparticles useful in various applications can make them toxic and harmful to the environment (Auffan et al. 2009; Bhatt and Tripathi 2011; Dinesh et al. 2012). Moreover, the concentrations of most metallic nanoparticles in the environment are unknown, but exposure modeling suggests that concentrations of gold nanoparticles in soil are higher than in water and air (Klain et al. 2008; Antisari et al. 2013). To date, the antimicrobial activity of gold nanoparticles has been extensively studied with human pathogenic bacteria under controlled conditions. However, it is essential to obtain the data of the eco-toxicological effect of gold nanoparticles on microorganisms that live in soil ecosystems under field conditions. This is very important, because it is well known that any change in soil community structure can have significant consequences for soil ecosystem processes. Changes in microbial activity and community composition can result from changes in availability of nutrients and/or anthropogenic activity and introduction of contaminants such as metallic nanoparticles. Soil microbial communities are responsible

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for many of the biogeochemical processes on Earth, such as nutrient mineralization and carbon/nitrogen cycling (Doran and Zeiss 2000). Therefore, many ecosystem services, including the quality of groundwater, waste degradation, and agricultural production, are dependent on the welfare of soil microbial biota. Soil microbial community structure and activities are difficult to explain using a single monitoring approach, therefore, for the better understanding of the soil microbial situation, different approaches need to be applied.

The aim of this work is to provide new data to evaluate the terrestrial ecotoxicity of the gold nanoparticles by performing different bioassays with microorganisms and soil enzymes involved in the most important biogeochemical cycles. To my knowledge this is the first reported investigation of the impact of the biogenic gold nanoparticles on garden soil processes.

Materials and methods

Streptomyces rimosus obtained from the Polish Culture of Microorganisms (PCM IAW116) was used for the formation of gold nanoparticles.

Formation and characterization of gold nanoparticles

The basal medium used in experiments consisted of (%): glycerol 0.8; KNO_3 0.01; $Na_2HPO_4 \times 2H_2O$ 0.0534; $MgSO_4 \times 7H_2O$ 0.05; KH_2PO_4 0.0272; microelements solution 1 mL ($FeSO_4 \times H_2O$ 0.1; $MnCl_2 \times 4\ H_2O$ 0.1; and $ZnSO_4 \times 7H_2O$ 0.1). The Erlenmeyer flasks were inoculated with bacterial cells and incubated at 28 °C with shaking (100 rpm) for 4 days. Then the biomass was filtered (Whatman filter paper No. 1) and extensively washed with distilled water to remove any medium component. Fresh and clean biomass (2 g) was taken into the Erlenmeyer flasks, containing 20 mL of Milli-Q deionised water (UV Ultrapure Water System, Burnstead, USA) and $HAuCl_4 \times 4H_2O$ (1 mM). The mixture was incubated with shaking at 50 °C for 18 h (in dark). Control (the gold ions without microbial biomass) was also run along with the experimental flasks. To isolate the gold nanoparticles, cells were washed twice with deionized water. Ultrasonic disruption of cells was carried out with an ultrasonic processor (TURBO 36800) over four to five 30 s periods and with an interval of 60 s between periods. The sonicated samples were centrifuged at 3500 rpm for 15 min at 4 °C to remove cell debris. The gold nanoparticles were separated by the sucrose density gradient technique described by Maliszewska (2013) and spheres concentrated in the 30 % fraction were studied. To verify reduction of gold ions, the solutions were scanned in the range of 200–800 nm in a spectrophotometer (UV-1650 PC, Shimadzu). The size and morphology of the nanoparticles were analyzed with the transmission electron microscope TEM (Zeiss EM 900). The sample was prepared by placing a drop of gold nanoparticles on a carbon-coated copper grid and subsequently drying in air before transferring it to the microscope. From electron micrographs the particle size distribution was found for at least 150 particles. The hydrodynamic diameter (size) and surface charge of the gold nanoparticles were studied through dynamic light scattering (DLS) measurements using a Zeta potential/particles sizer, NICOMP 380 ZLS, PSS.NICOMP Particle Sizing System at 25 °C. The nanoparticles were also analyzed in a scanning microscope with an energy-dispersive elemental (EDS) analyzer (SEM, JEOL JSM 5800 LV/EDS-ISIS 300-Oxford). For FTIR spectrum analysis, the gold nanoparticles synthesized were centrifuged at 14,000 rpm for 30 min to remove free proteins and other components present in the solution. The centrifuged, collected, and lyophilized particles were made in a KBr pellet, and the spectrum was recorded with an FTIR spectrometer (Perkin Elmer 1600). The stability study of the gold nanoparticles was carried out at room temperature. The change in surface plasmon resonance of the nanoparticle dispersion was recorded up to six months using UV–vis spectroscopy. The gold nanoparticles were lyophilized (Freeze Dryer Modulyo, Edwards) and their effect on soil biota was examined. The concentration of the gold nanoparticles was estimated from UV–Vis spectra according to the method described by Haiss et al. (2007).

Microcosm setup

The effect of gold nanoparticles synthesized by the biomass of *S. rimosus* PCM IAW116 on soil biota was performed according to the method described by Shah and Belozerova (2009) with a slight modification. Garden soil and potting mix were sieved through a 1.5 mm mill. Potting mix was added to the soil to the concentration of 6.0 % (w/w) to make the soil rich in nutrients and mimic the agricultural soil. The tested concentrations were selected according to the data obtained by Pereira et al. (2011) and Nogueira et al. (2012). The exposure doses of gold nanoparticles (16 and 33 mg kg^{-1} soil) were chosen to represent two scenarios: low and high effects. The gold nanoparticles were added to the soil in a beaker and samples were continuously stirred to prevent nanoparticle settling and to maintain homogeneous dispersions. In 250 mL Stericup Filter System Jars (Millipore, USA), 100 g of soil containing nanoparticles was introduced. These jars were closed with the supplied filters thereby allowing maintenance of stable humidity conditions but at the same time permitting aeration. The jars were incubated at 22±0.1 °C under static conditions. Additionally, one pattern without application of gold nanoparticles was performed as a control treatment. The study was carried out in triplicate. After 28 days of incubation, the soil subsamples were used for enumeration of culturable microorganisms and community level physiological analysis.

Enumeration of culturable soil microorganisms

The total number of culturable soil microorganisms was determined using the soil dilution plate-count technique. Hundredfold dilutions were prepared from the original soil suspension and the dilutions were plated on nutrient agar (NA), yeast malt extract agar (YMA), potato dextrose agar (PDA), and cold soil extract agar (CSEA). The plates were incubated at 25±0.1 °C and colonies were counted after 48 h of incubation. CSEA medium has the least organic content and favors growth of slow growing organisms, whereas PDA favors mainly fungi. NA and YMA favor the growth of bacteria and yeast, respectively.

The enumeration of the following bacteria: oligotrophic bacteria, copiotrophic bacteria, spore-forming oligotrophic bacteria and spore-forming copiotrophic bacteria were done on the Hattori's medium (Hattori and Hattori 1980); ammonifying bacteria, nitrogen-fixing bacteria and cellulose - decomposing bacteria – were done on the Zaborowska's medium (Zaborowska et al. 2006). The colony-forming units of *Azotobacter* spp. was numerated by the method described by Fenglerowa (1965). Determination of the number of *Arthrobacter* spp. was made on the Mulder and Antheumisse's medium (Mulder and Antheumisse 1963); *Pseudomonas* spp. – on the Mulder and Antheumisse's medium containing nystatin (Mulder and Antheumisse 1963). Actinomycetes were numerated using plate counts made on glycerol casein agar amended with 0.05 gL^{-1} cyclohexamine. The plates were incubated at 25–28 °C for 7–12 days.

Community Level Physiological Profiling (CLPP) analysis

Community level physiological profiles were obtained using Biolog™ Ecoplates (Biolog, Hayward, CA). Biolog EcoPlates, are 96-well plates, containing 31 different carbon sources plus a control well. Tetrazolium violet redox dye was used for each well as a colour indicator if added microorganisms utilized the substrates. One g portions of soil were shaken in 99 mL of distilled sterile water for 20 min at room temperature and then were incubated at 4±0.1 °C for 60 min. Next, 150 μL of each sample were inoculated into each well of Biolog™ EcoPlates and incubated at 25 °C. The rate of utilization was indicated by the reduction of the tetrazolium, a redox indicator dye that changes from colourless into purple. Data were recorded with a absorbance microplate reader Spectra Max® $Plus^{384}$ at 590 nm after 72 h. Microbial response in each microplate that expressed average well-colour development (AWCD) was determined as follows:

$$AWCD = \Sigma OD_{590}/31,$$

where OD_{590} is the optical density value from each well, corrected subtracting the blank well (inoculated, but without a carbon source) values from each plate well (Frąc et al. 2012).

Enzymatic activity determination

The soil was treated with the following concentrations of the gold nanoparticles: 16, 20, 25, 30, and 33 mg kg^{-1} soil, including the control samples where no gold nanoparticles were added to the soil. After 28 days of incubation, the activity of four enzymes was performed. Dehydrogenase activity (DEH) was determined as described by Casida et al. (1964) and the reddish colour intensity of the filtrate was measured with a spectrophotometer (UV-1650 PC, Shimadzu) at a wavelength of 485 nm. The results of the samples were compared with triphenyl formazan standards. Alkaline phosphatase and β-glucosidase activities were determined following the methods reported by Tabatabai and Bremner (1969), and Abellan et al. (2011), respectively. Urease activity was determined by staining the ammonium released into the incubation solution at 37 ±0.1 °C for two hours described by Kandeler and Gerber (1988) and Kandeler et al. (1999).

Microbial respiration study and carbon transformation test

In the respiration study, 80 g of soil sample was used, adjusting the water content to the 50 % of the soil water holding capacity. The soil was treated with the following concentrations of the gold nanoparticles: 16, 20, 25, 30, and 33 mg kg^{-1} soil, including the control samples where no gold nanoparticles were added to the soil. These samples were incubated in manometric respirometers, which allow the determination of the sample oxygen consumption (OxiTop® system WTW GmbH, Weilheim, Germany). Basal respiration rate (*BR*) was estimated as the average hourly respiration rate over the last 5 days of incubation when the respiration was stable. Cumulative respiration (*CR*) was determined by the cumulative oxygen consumption at the end of the incubation period. After 28 days of incubation, glucose-induced respiration (*GIR*) was determined according to OECD 217 carbon transformation test guidelines (OECD 2000b). This experimental test was done by adding an aqueous solution equivalent to 33 mg glucose per kg of soil to the incubated samples and the total quantities of oxygen consumed during the 12 hours were measured and mean respiration rates were determined. The respiratory activation quotient (Q_R) was calculated dividing *BR* by *GIR* (ISO 2002).

Nitrogen transformation test

The nitrogen transformation test was performed according to the OECD 216 guidelines (OECD 2000a). The soil was treated with the following concentrations of the gold nanoparticles: 16, 20, 25, 30, and 33 mg kg^{-1} soil, including the control samples when no gold nanoparticles were added to the soil. The concentration of the nitrates after 28 days was determined

by the modified brucine method (Nagaraja et al. 2003). The data from these tests are used to prepare a dose–response curve and calculate EC_{50} value (EC_{50}=the concentration of gold nanoparticles in soil that results in a 50 percent inhibition of nitrogen transformation). The EC_{50} was selected, because it is the most available in the literature's parameters used in the environmental regulations.

Chemical analysis of the soil

Soil Total Organic Carbon (TOC) was measured using the EPA 415.1 method, whereas Total Kjeldahl Nitrogen (TKN) was measured using the EPA 315.3 method. Soil pH was determined using a soil: water ratio of 1:10 with a glass electrode.

Statistical analysis

All experiments were run in triplicate. The statistical analyses have been performed using the statistical suite StatSoft. Inc. (2011) STATISTICA (data analysis software system) version 10.0 and Excel. The quantitative variables were characterized by the arithmetic mean of standard deviation or median or max/min (range) and the 95 % confidence interval. Statistical significance of differences between two groups was processed with the Student's t test. In all the calculations, a *P*-value of 0.05 was used as the cut-off for statistical significance.

Results

Formation and characterization of gold nanoparticles

The gold nanoparticles used in this work were synthesized by the incubation of biomass of *S. rimosus* PCM IAW116 with gold ions. Formation of gold nanoparticles is easily identifiable due to the change in the colour of the biomass (inset in Fig. 1a). The excitation spectrum of these particles was characterized by UV-visible spectroscopy analysis. The UV–vis spectrum showed the presence of a single plasmon band at 536 nm which is assigned to spherical nanoparticles (Fig. 1a). Further evidence for the formation of gold nanoparticles was provided by SEM-EDS analysis. The strong signals corresponding with elemental gold together with signals from C, N, O, K, Ca, Fe, Si and Cl are shown in Fig. 1b. The C, O, N signals may arise from proteins, which are bound to the gold nanoparticles. The Si, K, Fe, Ca, and Cl signals are likely caused by X-ray emission from the glass substrate and salts in the vicinity of the gold nanoparticles.

TEM and DLS techniques were used to determine the morphology and size of the nanoparticles. Figure 1c shows a representative TEM micrograph of the nanoparticles studied. These particles are spherical in shape and uniformly distributed without any significant agglomeration. The particle size histogram (Fig. 1d) shows that the nanoparticles sizes range from 10 to 22 nm and possess an average size of 14± 3 nm. The frequency of distribution observed from the histogram indicates that almost 60 % of the gold particles are in the 10- to 13-nm sized range. It should be noted that the particle size obtained from dynamic light scattering measurements is higher than that estimated from TEM measurements and is 22 ±3 nm. This is due to the fact that the overall particle size so obtained is augmented substantially by contributions from the hydrated capping agents (proteins) and also from solvation effects. The zeta potential of the biogenic gold nanoparticles showed the value of −33±3 mV, indicating the negative charge and great stability of these particles in the aqueous suspension. To investigate the stability of gold nanostructures, the particles were stored at room temperature for the period of 6 months. Any precipitation was not observed even after 6 months of storage suggesting that these colloidal gold nanoparticles are extremely stable (data not shown). Such long-term stability of the particles indicated that nanostructures are stabilized in the solution by the capping agent, which is likely to be protein(s). The Fourier transform infrared spectroscopy measurements were carried out to examine the molecules that are responsible for the stability of synthesized nanoparticles. Figure 2 shows the Fourier transform infrared spectrum of gold nanoparticles reduced by the biomass of *S. rimosus* PCM IAW116. Intense absorptions are observed at 3355.7 cm^{-1}, 2923.06 cm^{-1}, 1652.26 cm^{-1}, 1543.7 cm^{-1} and 1058.87 cm^{-1}. The IR bands at ~3355 cm^{-1} and ~2923 cm^{-1} are characteristic of the stretching vibration of OH or NH groups. The amide I and II bands of proteins are expected to occur as prominent IR bands around 1660 cm^{-1} and 1535 cm^{-1}, respectively (Philip 2009). In this case, the intense bands observed at 1652 cm^{-1} and 1543 cm^{-1} arise from the amide I and II. The amide band I is assigned to the stretch mode of the carbonyl group coupled to the amide linkage, while the amide II band arises as a result of the N-H stretching molecules of vibration in the amide linkage (Kathiresan et al. 2010). The band at approximately 1058 cm^{-1} corresponds to C-N stretching vibrations of amine (Narayanan and Sakthivel 2008). This clearly indicates that amine groups of proteins may be responsible for stabilization of the gold nanoparticles synthesized by the biomass of *S. rimosus*.

Soil chemical properties

Soil pH value at the start of the experiment was neutral (7.3), and remained stable during the experimental period (28 days; pH 7.55); the same was true for soil organic C and N (TOC and TN) content (Table 1).

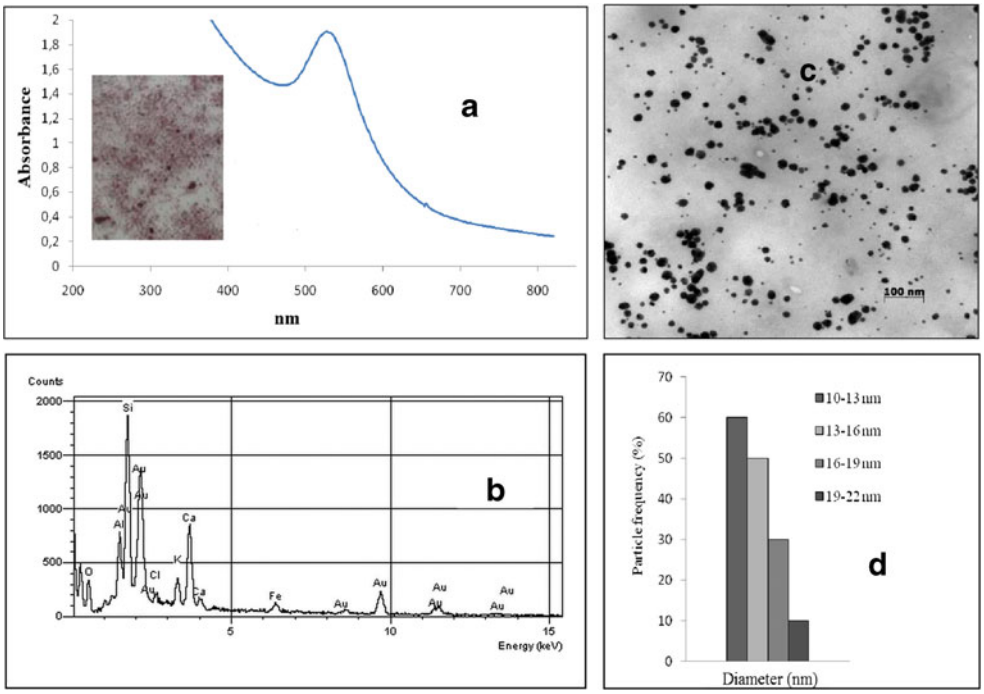

Fig. 1 UV–vis spectrum of the gold nanoparticles synthesized by the reacting of biomass of *S. rimosus* with gold ions; the inset shows the colour of the biomass after incubation with gold ions (**a**); EDS profile of the gold nanoparticles (**b**); the representative TEM image of the gold nanoparticles synthesized by the reacting of biomass with gold ions (**c**); histogram of the gold nanoparticles size distribution obtained from TEM (**d**)

Influence of the gold nanoparticles on soil microbial community structure

Actually, there are no standard methodologies for evaluating the effects of exposure of metallic nanoparticles on microbial biota in soil. Therefore, microcosms for studying the influence of the gold nanoparticles synthesized by the biomass of *S. rimosus* PCM IAW116 on the soil microbial community was used. Microcosms have been frequently applied as direct analogs of ecological systems in studying trophic interactions by offering the controlled environment to research the interactions. There have been numerous reports in this area, and it may not be necessary to list them all here. As can be seen in Table 1, a decrease in the number of colonies formed on CSEA, NA, PDA, and YMA is observed for the soil samples containing the gold nanoparticles synthesized by the biomass of *S. rimosus* PCM IAW116. However, there are no statistically significant differences in the number of colony forming units between the control soil samples and those exposed to nanoparticles (*P* values for all cases were greater than 0.05). No significant difference was also observed for total plate counts of the studied soil microbial groups, i.e., copiotrophic bacteria, spore-forming copiotrophic bacteria, oligotrophic bacteria, spore-forming oligotrophic bacteria, ammonifying bacteria, nitrogen immobilizing bacteria, cellulose-decomposing bacteria, *Azotobacter* spp., *Arthrobacter* spp., *Pseudomonas* spp., and actinomycetes (Table 1, *P* values for all cases was higher than 0.05).

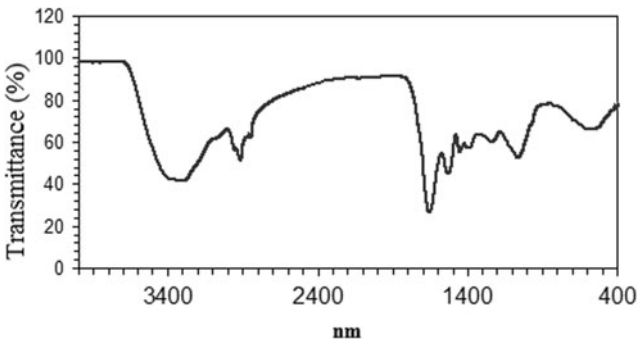

Fig. 2 FTIR spectrum recorded from powder of the gold nanoparticles

Analysis of the ratio of different taxonomic/ecological groups of microorganisms in the control soil sample and those exposed to the biogenic gold nanoparticles indicates an interesting change in microbial community structure. There was noted a slight decline in the ratio of copiotrophic bacteria and spore-forming copiotrophic bacteria to oligotrophic bacteria and spore-forming oligotrophic bacteria ($Cop+Cop_{sf}$ / $Olig+Olig_{sf}$). The increase in the ratio of *Azotobacter* spp. to ammonifying bacteria (Am) was also observed. The populations of *Actinomycetes* and *Arthrobacter* spp. were also increased when the soil had been enriched with the biogenic gold nanoparticles.

The functional diversity (catabolic potential) was assessed using Average Well Color Development (AWCD). This index was calculated following the community level physiological profiling (CLPP) using Biolog™ Eco Plates. The values of the AWCD index from the soil sample incubated with the gold nanoparticles and control soil samples (without nanoparticles) showed no significant differences (Table 1, *P* values for all cases was higher than 0.05).

Table 1 Chemical and biological analysis of soil samples incubated with and without the studied gold nanoparticles

Parameter	Control 0 day	Control 28 days	With Au, 28 days	
			16 mg kg^{-1} soil	33 mg kg^{-1} soil
TOC (mg kg^{-1})	16.44	15.87	16.07	16.09
TKN (mg kg^{-1})	3.45	3.21	3.3	3.36
pH	7.3	7.45	7.55	7.55
C:N	4.8	4.9	4.8	4.8
[a] in CSEA	4120±42.5	3850±47.4	3230±41.7	3170±31.7
[a] in NA	5900±60.7	5450±59.3	4740±55.5	4820±47.9
[a] in PDA	1860±28.9	1740±25.1	1000±18.6	1120±15.9
[a] in YMA	5670±51.7	4920±55.3	4110±46.6	4310±44.4
Cop [a]	690±9.8	670±7.5	560±6.8	580±6.1
Cop_{sf} [a]	210±6.5	200±7.5	250±5.9	240±7.3
Olig [a]	550±9.4	530±8.1	510±4.8	520±7.8
$Olig_{sf}$ [a]	80±6.7	73±7.1	82±5.8	83±8.9
Am [a]	3100±37.7	3000±35.8	2780±35.5	2650±36.5
Im [a]	2850±33.8	2790±36.7	2600±38.1	2670±35.5
*Azotobacte*r spp. [a]	25±4.4	21±3.7	24±2.9	23±5.2
Cel [a]	124±18.2	115±11.6	119±11.4	115±12.4
Arthrobacter spp. [a]	4100±53.7	4120±51.5	4210±53.4	4050±44.9
Pseudomonas spp. [a]	3510±49.6	3470±35.6	3470±32.6	3300±32.5
Actinomycetes [a]	2560±31.8	2510±39.5	2550±33.3	2495±31.9
AWCD	1.18±0.03	1.19±0.03	1.21±0.04	1.23±0.07

[a] The results are presented in 10^3 CFU g^{-1} of dry weight (±standard deviation, n=3)

Cop-copiotrophic bacteria; Cop_{sf} - spore-forming copiotrophic bacteria; Olig- oligotrophic bacteria; $Olig_{sf}$ - spore-forming oligotrophic bacteria; Am- ammonifying bacteria; Im- nitrogen fixing bacteria; Cel-cellulose-decomposing bacteria

Data in Fig. 3 summarize the results of enzyme assays for soils incubated in the presence of the gold nanoparticles synthesized by *S. rimosus* PCM IAW116. There are some variations, especially with dehydrogenase (DEH), but when compared to the soil control, it was found there were negligible differences between the activity of enzymes in the soil treated with the biogenic gold nanoparticles and the control ($P>0.05$).

Soil respiration was measured to examine the effects of different doses of the studied gold nanoparticles on overall microbial activity. The graph in Fig. 4 shows the cumulative respiration curves obtained from 28-day monitoring of O_2 consumption by the soil microbial community. The effect of the gold nanoparticles on respiration of soil microorganisms was determined from parameters and profile of those curves. As can be seen from Fig. 4, there are no substantial impacts on soil respiration from the biogenic gold nanoparticles at any concentration (none of the studied concentrations of the gold nanoparticles exhibit values of accumulated oxygen below the control). After 28 days of incubation, the cumulative oxygen consumption (*CR*) was 493±11.9 mg of O_2 per kg of dry weight for control and 509±11.1, 532±13.3, 541±19.2. 547,±20.3 and 553±21.9 mg of O_2 per kg of dry weight for soil containing 16, 20, 25, 30, and 33 mg g^{-1} of gold nanoparticles, respectively.

Table 2 shows the values of % *CR, BR*, *GIR,* and Q_R for the gold nanoparticles synthesized by the biomass of *S. rimosus*. The *CR* reflects the overall soil state (microbiota and nutrient availability) and these results show that up to the concentration of 33 mg kg^{-1} there is no significant difference ($P>0.05$) between the control samples and the soil treated with the gold nanoparticles. An insignificant difference ($P>0.05$) in the values of *BR*, *GIR*, and Q_R is shown in Table 2. Also, the gold nanoparticles up to the concentration of 33 mg kg^{-1} soil do not affect the nitrifying microbiota. The value of EC_{50} (in mg kg^{-1} soil) calculated from the dose–response curve in the nitrogen transformation test is above 100 mg kg^{-1} (data not shown).

Discussion

In this work, a simple biosynthesis method was used to produce monodispersed colloidal gold nanoparticles. A number of approaches are available for the synthesis of gold nanoparticles. They can be easily synthesized and separated by

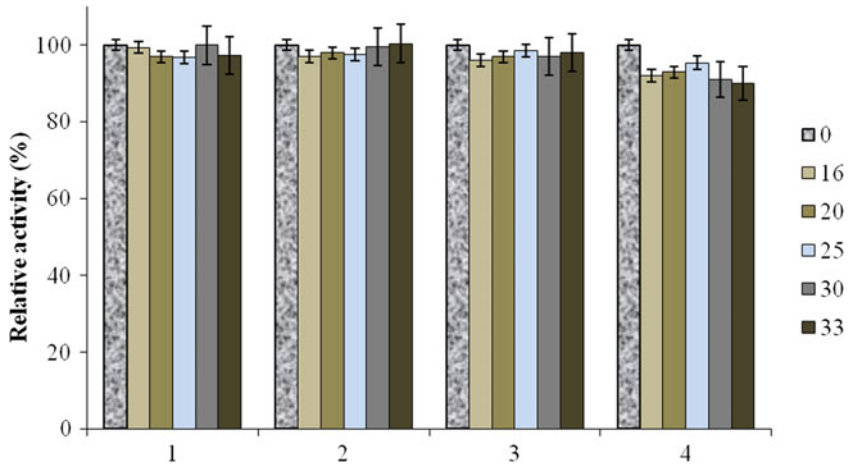

Fig. 3 The relative activity [%] of β-glucosidase (1), urease (2), alkaline phosphatase (3), and dehydrogenase (4) for soil samples containing different concentrations of the gold nanoparticles in mg kg^{-1} dry soil

various chemical/physical methods (Guo and Wang 2007; Huang et al. 2009) and biological techniques. Among these methods, biological techniques are not only a good way to fabricate benign nanostructures but also to reduce the use or generation of hazardous substances to human health and the environment. A number of previous reports (Maliszewska 2011; Nath and Banerjee 2013; Priyadarshini et al. 2013) have demonstrated that microbes can be considered as a "natural nano-factory" systems.

The colloidal gold nanoparticles used in these experiments are extremely stable and showed no aggregation even after storage for 6 months (data not shown). Such long-term stability of these nanostructures indicated that nanoparticles are stabilized by the capping agent, which is likely to be proteins. These findings are in agreement with several previous reports, which showed that interactions between amino acids and metal ions might be responsible for the stabilization of gold nanoparticles (Nath and Banerjee 2013; Prem et al. 2013; Priyadarshini et al. 2013; Quester et al. 2013).

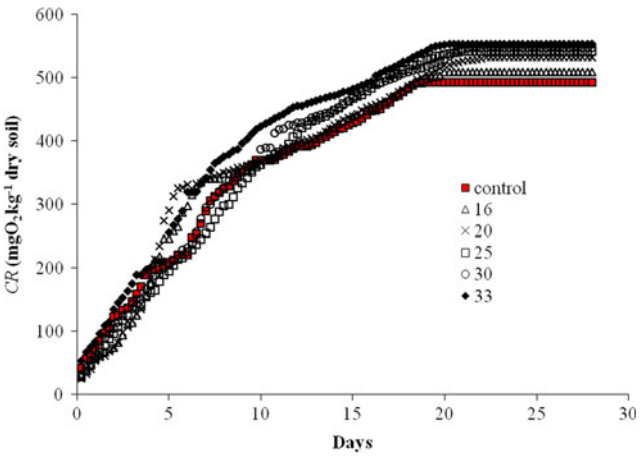

Fig. 4 Values of the cumulative respiration expressed as the cumulative oxygen consumed during 28 days of the respirometric assay for the control and different concentrations of the gold nanoparticles in mg kg^{-1} dry soil. The values correspond to the averages of at the least three replicates

The pollution of the garden soil with the biogenic gold nanoparticles did not change any chemical and physical property of soil (e.g., pH, total organic C, total N) as it happens in the case of heavy metals pollution of soil (Chander et al. 1995).

The impact of the studied gold nanoparticles on the microbial communities structure and soil processes was analyzed. Five different experimental techniques were used to assess the potential effects of the gold nanoparticles released into the environment. These procedures were found to be: (i) enumeration of culturable soil microorganisms (soil microbial community structure), (ii) community level physiological profiling (CLPP) analysis, (iii) determination of soil enzymatic activities (iv) soil respiration and (v) carbon/nitrogen transformation. The enumeration of culturable soil microorganisms and CLPP analysis were used to define the effects of gold nanoparticles on the size and structure of the microbial community. Community level physiological profiles based on carbon substrate utilization patterns is considered a rapid and reproducible technique to measure microbial functional diversity (Garland and Mills 1991), although the technique has several drawbacks such as culture-dependence and the possibility of changes in the microbial community during the incubation (Ros et al. 2008). The responses of soil CLPP can mirror

Table 2 Average rate of cumulative respiration (*CR*) during the incubation period expressed as the % relative to the control soil; *BR* (mg O_2 h^{-1} kg^{-1} dry soil), *GIR* (mg O_2 h^{-1} kg^{-1} dry soil), and Q_R

Parameter	Concentration of the biogenic gold nanoparticles (mg kg^{-1})					
	0	16	20	25	30	33
% CR	100.0	103.3	108.1	110.0	111.2	112.4
BR	1.34	1.32	1.31	1.33	1.38	1.41
GIR	7.65	7.76	7.70	7.82	7.26	6.71
Q_R	0.18	0.17	0.17	0.17	0.19	0.21

% CR - percentage of cumulative respiration, *BR* - basal respiration rate, *GIR*=glucose- induced respiration, Q_R - respiratory activation quotient

changes in microbial biomass (Johnson et al. 1998). Based on the obtained results, it was established there is a statistically insignificant influence of the gold nanoparticles synthesized by the biomass of *S. rimosus* PCM IAW116 on the number of colony forming units and the AWCD index. These results are in agreement with the observations of Shah and Belozerova (2009) but contradict the data shown by Nogueira et al. (2012) and Galindo et al. (2013). Shah and Belozerova (2009) studied the effect of gold nanoparticles on the microbial community, after an exposure period of 15 days. In this work, the gold particles did not cause significant effects on soil biota, both in the number of colony forming units, metabolic fingerprinting (BIOLOG), and in FAME. However, no data was provided about the size, the surface properties, and charge of particles tested. Nogueira et al. (2012) evaluated the effect of the gold nanorods with a small average size (12.32 nm) and the positive surface charge (24.8 mV) on the microbial community. This study reinforces deep changes in the structural diversity of the microbial community, analyzed by PCR-DGGE profiles. Galindo et al. (2013) analyzed the effect of chemically synthesized gold nanorods possessing an average size of 12.3 nm and the positive surface charge 24.8 mV on the growth of white-rot fungi species *Trametes versicolor*, *Lentinus sajor caju*, *Pleurotus ostreatus*, and *Phanerochaete chrysosporium*. According to the results obtained by Galindo et al. (2013) and for the classification of the European Union Commission Guideline 93/67/EEC-Annex 1 (1996), the analyzed gold nanoparticles should be placed in the category of harmful.

It was observed there were not significant changes in microbial community structure related to the ratio of copiotrophic bacteria and spore-forming copiotrophic bacteria to oligotrophic bacteria and spore-forming oligotrophic bacteria ($Cop+Cop_{sf}$ / $Olig+Olig_{sf}$) and *Azotobacter* spp. to ammonifying bacteria (Am). It also changed the population of *Actinomycetes* and *Arthrobacter* spp. Such minor amendments in the community structure of the soil microbes indicate that prolongated exposure of soil microbes to gold nanoparticles is the beginning of change in the population of soil microorganisms. Studying the impact of various metallic nanoparticles on microorganisms in the environment is therefore justified.

As shown above, gold nanoparticles have been reported in the literature as both nontoxic and toxic to the microbial community. The controversy may arise from details in the material preparation or chemical/colloidal stability of the nanoparticles. For example, it was also observed that the increase in cytotoxicity is mainly due to the aggregation, which is determined by such factors as the number of particles, surface properties, and biological process (Diegoli et al. 2008; Yen et al. 2009; Radada et al. 2012; Baumann et al. 2014; Merdzan et al. 2014). Moreover, some authors demonstrated that the toxicity of the gold nanoparticles is most often higher for positively charged nanostructures due to better biocompatibility to the negatively charged cellular lipid bilayer (Goodman et al. 2004).

It is well known that soil enzymatic activities are good indicators to assess both beneficial and harmful effects on soil health (Tong et al. 2007). In this study, there was examined the activity of soil β-glucosidase, urease, and alkaline phosphatase, which play an essential role in C, N, and P cycles. Dehydrogenase activity (as an indicator of oxidative capacity of soil microorganisms) was also evaluated (Sukul 2006). The reduction in the enzyme activity would be expected in a system exposed to a toxic or interfering material. The obtained results showed that the enzymatic activities have not been adversely affected by the gold nanoparticles at any concentration tested. It seems that dehydrogenase activity is one of the most sensitive parameters among the studied enzymatic activities. This enzyme was previously used as a good indicator of soil ecotoxicological test caused by heavy metals (Maliszewska-Kordybach and Smeeczak 2003; Hinojosa et al. 2004). It is important to mention that no data on the effect of gold nanoparticles on soil enzymatic activities have been found in the literature to compare with the results in this work, except a study that showed that silver nanoparticles have no influence on the soil enzymes (Hänsch and Emmerling 2010).

The impact of the nanoparticles on soil microbial function was evaluated by the OxiTop® instrument. The determination of oxygen consumption has been an internationally used and accepted standard method as changes in this parameter are a direct result of microbial metabolism. An unquestionable attribute of this method is the high data density providing by the continuously oxygen consumption recording (360 values in a period of 28 days). A reduction in the rate of respiration compared to the control would be expected if soil populations were subjected to a toxic material (Jones and Ananyeva 2001; Tong et al. 2007). In the case of the gold nanoparticles studied, it was confirmed that these particles have no toxicity towards soil microbiota, reflected as uninterrupted respiratory activity of the soil. The *CR* shows the overall soil state (microbiota and nutrient availability). Basal respiration rate (*BR*) indicates the current biological activity, which remains constant after the soil respiration is stabilized. Considering that the same soil was used in all of the experiments, the nutritional state is the same for all of the samples, and the variable is the microbiota state, the results for the *BR* may indicate if the analyzed contamination damages the soil microorganisms (Peric et al. 2014). The obtained values of *BR* clearly confirmed that there is no toxic effect of the examined gold nanoparticles on the soil biota.

According to the ISO standards for the determination of activity of soil microorganisms using respiration curves, the values of $Q_{R,}$ which if higher than 0.30 indicate polluted soil, especially in the case of heavy metals (ISO 2002). When the gold nanoparticles formed by the biomass of *S. rimosus* were introduced into the soil, the calculated values of Q_R are not

higher than 0.21. Moreover, according to the results for nitrogen transformation processes and to the Globally Harmonized System of classification and labelling of chemicals (EC_{50}> 100 mg kg^{-1}) (United Nations 2006) there is no toxic effect of the analyzed gold nanoparticles on the nitrifying microbiota.

It should be noted that no data on the toxicological effects of gold nanoparticles on soil respiration have been found in the literature to compare with the results obtained in this study. García et al. (2012) investigated the effect of the spherical gold nanoparticles possessing an average size of 20 nm and the negative surface charge −39.2 mV on the respiratory activity of the microbial communities but present in a modern wastetreatment plant. Specifically, the action of ordinary heterotrophic organisms (OHO), ammonia oxidizing bacteria (AOB), thermophilic and mesophilic anaerobic bacteria were studied. The results showed that the gold nanoparticles had no significant effect on the OHO sludge. In contrast to OHO biomass, an inhibitory effect (around 14 %) on the AOB was detected. Authors underlined that it was a low inhibition, and it did not increase with exposure time. Anaerobic biogas production tests were carried out for the gold nanoparticles studied in the presence of mesophilic and thermophilic communities of anaerobic population from large-scale anaerobic digesters. No statistical differences were found among all the experiments studied for either mesophilic or thermophilic conditions. These results clearly leads to the inference that the gold nanoparticles did not cause significant effects on microbial communities intended for wastewater treatment.

Conclusions

In conclusion, the terrestrial ecotoxicity of the gold nanoparticles synthesized by the biomass of *S. rimosus* was analyzed. Based on the presented results, it was concluded that these gold nanoparticles can be classified as "not harmful." It should be noted that it is far too early to conclude from this work that the gold nanoparticles formed by microbes and released into the soil do not influence the soil biota in any way. Data are necessary for the long-term effects of gold nanoparticles on soil microbial populations in a range of soils with varying physico-chemical characteristics and soils from different ecosystems. Moreover, in the light of the results and discrepancies obtained in this and other works with gold nanoparticles, it is evident that, when reporting the toxicity effects of nanoparticles, it is essential to describe the characterization parameters, such as surface charge and presence of stabilizers.

In spite of the fact that some microbes are particularly useful, not only in accumulation of metals, but also in the formation of stable metallic nanoparticles under normal air pressure and at room temperature, there is no industrial technology for their preparation using living organisms. I am convinced that evaluation of the impact of biogenic-gold nanoparticles on soil microorganisms is an important step to find justification for the biological synthesis of nanoparticles on an industrial scale.

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References

Abellan MA, Baena CW, Morote FAG, Cordoba MIP, Perez DC, Lucas-Borja ME (2011) Influence of the soil storage method on soil enzymatic activities in Mediterranean forest soils. For Syst 20:379–388

Antisari LV, Carbone S, Gatti A, Vianello G, Nannipieri P (2013) Toxicity of metal oxide (CeO_2, Fe_3O_4, SnO_2) engineered nanoparticles on soil microbial biomass and their distribution in soil. Soil Biol Biochem 60:87–94

Auffan M, Rose J, Bottero JY, Lowry GV, Jolivet JP, Wiesner MR (2009) Towards a definition of inorganic nanoparticles from an environmental, health and safety perspective. Nat Nanotechnol 4:634–641

Baumann J, Köser J, Arndt D, Filser J (2014) The coating makes the difference: Acute effects of iron oxide nanoparticles on *Daphnia magna*. Sci Total Environ 484:176–184

Bhatt I, Tripathi BN (2011) Interaction of engineered nanoparticles with various components of the environment and possible strategies for their risk assessment. Chemosphere 82:308–317

Casida L Jr, Klein D, Santoro T (1964) Soil dehydrogenase activity. Soil Sci 98:371–377

Chander K, Brookes PC, Harding SA (1995) Microbial biomass dynamics following addition of metal-enriched sewage sludges to a sandy loam. Soil Biol Biochem 27:1409–1421

Diegoli S, Manciulea AL, Begum S, Jones IP, Lead JR, Preece JA (2008) Interaction between manufactured gold nanoparticles and naturally occurring organic macromolecules. Sci Total Environ 402:51–61

Dinesh R, Anandaraj M, Srinivasan V, Hamza S (2012) Engeenered nanoparticles in the soil and their potential implications to microbial activity. Geoderma 173–174:19–27

Doran JW, Zeiss MR (2000) Soil health and sustainability: managing the biotic component of soil quality. Appl Soil Ecol 15:3–11

Fenglerowa W (1965) Simple method for counting *Azotobacter* in soil samples. Acta Microbiol Pol 14:203–206

Frąc M, Oszust K, Lipiec J (2012) Community level physiological profiles (CLPP), characterization and microbial activity of soil amended with dairy sewage sludge. Sensors 12:3253–3268

Galindo TPS, Pereira R, Freitas AC, Santos-Rocha TAP, Rasteiro MG, Antunes F, Rodrigues D, Soares AMVM, Gonçalves F, Duarte AC, Lopes I (2013) Toxicity of organic and inorganic nanoparticles to four species of white-rot fungi. Sci Total Environ 458–460:290–297

García A, Delgado L, Torŕ JA, Casals E, González E, Puntes V, Font X, Carrera J, Sánchez A (2012) Effect of cerium dioxide, titanium dioxide, silver, and gold nanoparticles on the activity of microbial communities intended in wastewater treatment. J Hazard Mater 199–200:64–72

Garland JL, Mills AL (1991) Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole carbon source utilization. Appl Environ Microbiol 57:2351–2359

Goodman CM, McCusker CD, Yilmaz T, Rotello VM (2004) Toxicity of gold nanoparticles functionalized with cationic and anionic side chains. Bioconjug Chem 2004(15):897–900

Guo SE, Wang E (2007) Synthesis and electrochemical applications of gold nanoparticles. Anal Chim Acta 598:181–192

Haiss W, Thanh NT, Aveyard J, Fernig DG (2007) Determination of size and concentration of gold nanoparticles from UV–vis spectra. Anal Chem 79:4215–4221

Hänsch M, Emmerling C (2010) Effects of silver nanoparticles on the microbiota and enzyme activity in soil. J Plant Nutr Soil Sci 173: 554–558

Hattori R, Hattori T (1980) Sensitivity to salts and organic compounds of soil bacteria isolated on diluted media. J Gen Appl Microbiol 26:1–14

Hinojosa MB, Carreira JA, García-Ruíz R, Dick RP (2004) Soil moisture pre-treatment effects on enzyme activities as indicators of heavy metal-contaminated and reclaimed soils. Soil Biol Biochem 36:1559–1568

Hristozov D, Malsch I (2009) Hazards and risks of engineered nanoparticles for the environment and human health. Sustainability 1:1161–1194

Huang X, Neretina S, El-Sayed MA (2009) Gold nanorods: from synthesis and properties to biological and biomedical applications. Adv Mater 21:4880–4910

ISO (2002) ISO guideline 17155: Soil Quality. Determination of Abundance and Activity of Soil Microflora Using Respiration Curves. International Organization for Standardization, Geneva

Johnson D, Leake JR, Lee JA, Campbell CD (1998) Changes in soil microbial biomass and microbial activities in response to 7 years simulated pollutant nitrogen deposition on a heathland and two grasslands. Environ Pollut 103:239–250

Jones WJ, Ananyeva ND (2001) Correlations between pesticide transformation rate and microbial respiration activity in soil of different ecosystems. Biol Fertil Soils 33:477–483

Kandeler E, Gerber H (1988) Short-term assay of soil urease activity using colorimetric determination or ammonium. Biol Fertil Soils 6:68–72

Kandeler E, Stemmer M, Klimanek EM (1999) Response of soil microbial biomass, urease and xylanase within particle size fraction to long-term soil management. Soil Biol Biochem 31:205–211

Kathiresan K, Alikunhi NM, Pathmanaban SM, Nabikhan A, Kandasamy S (2010) Analysis of antimicrobial silver nanoparticles synthesized by coastal strains of *Escherichia coli* and *Aspergillus niger*. Can J Microbiol 56:1050–1059

Klain SJ, Alvarez PJJ, Batley GE, Fernandes TF, Handy RD, Lyon DY, Mahendra S, McLaughlin MJ, Lead JR (2008) Nanomaterials in the environment: behavior, fate, bioavailability, and effects. Environ Toxicol Chem 27:1825–1851

Maliszewska I (2011) Microbial synthesis of metal nanoparticles. In: M. Rai M & Duran N (ed) Metal nanoparticles in microbiology Springer-Verlag Berlin Heidelberg, p 153–176

Maliszewska I (2013) Microbial mediated synthesis of gold nanoparticles: preparation, characterization and cytotoxicity studies. Dig J Nanomater Bios 8:1123–1131

Maliszewska-Kordybach B, Smeeczak B (2003) Habitat function of agricultural soils as affected by heavy metals and polycyclic aromatic hydrocarbons contamination. Environ Int 28:719–728

Merdzan V, Domingos RF, Monteiro CE, Hadiou M, Wilkinson KJ (2014) The effects of different coatings on zinc oxide nanoparticles and their influence on dissolution and bioaccumulation by the green alga, *C. reinhartii*. Sci Total Environ 488–489:316–324

Mulder EG, Antheumisse J (1963) Morphologie, physiologie et ecologie des *Arthrobacter*. Ann Inst Pasteur 105:46–74

Nagaraja P, Kumar MH, Yathirajan H, Prakash J (2003) Highly sensitive reaction of nitrate with brucine and 3-methyl-2-benzothiazolinone hydrazone hydrochloride for the determination of nitrate in environmental samples. Anal Sci 19:961–963

Narayanan KB, Sakthivel N (2008) Coriander leaf mediated biosynthesis of gold nanoparticles. Mater Lett 62:4588–4590

Nath D, Banerjee P (2013) Green nanotechnology – a new hope for medical biology. Environ Toxicol Pharmacol 36:997–1014

Nogueira V, Lopes I, Rocha-Santos T, Santos AL, Rasteiro GM, Antunes F, Gonçalves F, Soares AM, Cunha A, Almeida A, Gomes NC, Pereira R (2012) Impact of organic and inorganic nanomaterials in the soil microbial community structure. Sci Total Environ 424:344–350

OECD (2000a) Guideline for Testing of Chemicals 216, Soil Microorganisms: Nitrogen Transformation Test. Organization for Economic Cooperation and Development, Paris

OECD (2000b) Guideline for Testing of Chemicals 217, Soil Microorganisms: Carbon Transformation Test. Organization for Economic Cooperation and Development, Paris

Pereira R, Rocha-Santos TAP, Antunes FE, Rasteiro MG, Ribeiro R, Gonçalves F, Soares AM, Lopes I (2011) Screening evaluation of the ecotoxicity and genotoxicity of soils contaminated with organic and inorganic nanoparticles: the role of ageing. J Hazard Mater 194: 345–354

Peric B, Sierra J, Martí E, Cruañas R, Garau MA (2014) A comparative study of the terrestrial ecotoxicity of selected protic and aprotic ionic liquids. Chemosphere 12, doi: 10.1016/j.chemosphere.2014.02.043

Philip D (2009) Honey mediated green synthesis of gold nanoparticles. Spectrochim Acta A 73:650–653

Prem LK, Kumar S, Srivastava AK, Sharma AK (2013) Myconanotechnology in agriculture: a perspective. World J Microbiol Biotechnol 29:191–207

Priyadarshini E, Pradhan N, Sukla LB, Panda PK, Mishra BK (2013) Biogenic synthesis of floral-shaped gold nanoparticles using a novel strain, *Talaromyces flavus*. Ann Microbiol. doi:10.1007/s13213-013-0744-4

Quester K, Avalos-Borja M, Castro-Longoria E (2013) Biosynthesis and microscopic study of metallic nanoparticles. Micron 54–55:1–27

Radada K, Al-Shraimb M, Moldzioc R, Rauschc WD (2012) Recent advances in benefits and hazards of engineered nanoparticles. Environ Toxicol Pharmacol 34:661–672

Ros M, Goberna M, Pascual JA, Klammer S, Insam H (2008) 16S rDNA analysis reveals low microbial diversity in community level physiological profile assays. J Microbiol Methods 72:221–226

Sadowski Z, Maliszewska I (2011) Applications of gold nanoparticles : current trends and future prospects. In: M. Rai M and Duran N (ed) Metal Nanoparticles in Microbiology. Springer-Verlag Berlin Heidelberg, p 225–248

Shah V, Belozerova I (2009) Influence of metal nanoparticles on the soil microbial community and germination of lettuce seeds. Water Air Soil Pollut 197:143–148

Sperling RA, Gil PR, Zhang F, Zanella M, Parak WJ (2008) Biological applications of gold nanoparticles. Chem Soc Rev 37:1896–1908

Sukul P (2006) Enzymatic activities and microbial biomass in soil as influenced by metalaxyl residues. Soil Biol Biochem 38:320–326

Tabatabai MA, Bremner JM (1969) Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. Biol Biochem 1:301–307

Tong Z, Bischoff M, Gnies L, Applegate B, Turco RF (2007) Impact of fullerene (C_{60}) on a soil microbial community. Environ Sci Technol 41:2985–2991

United Nations (2006) Globally Harmonized System of classification and labeling of chemicals. UN/SCEGHS/12/INF5. New York, USA

Yen HJ, Hsu SH, Tsai CL (2009) Cytotoxicity and immunological response of gold and silver nanoparticles of different sizes. Small 13: 1553–1561

Zaborowska M, Wyszkowska J, Kucharski J (2006) Microbial activity in zinc contaminated soil of different pH. Pol J Environ Stud 15:569–574