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Comparative effect of silver nanoparticles on maize rhizoplane microbiome in initial phase of plants growth**

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Abstract. The aim of the experiments was to evaluate shifts in the prokaryotic and eukaryotic microbiome of maize rhizoplanes treated with five forms silver nanoparticles with different surface properties, produced by chemical reduction of silver(V) nitrate. Metagenomic studies were performed using appropriate procedures to create NGS libraries and sequences to species. All silver nanoparticles forms used moderately limited the growth of maize, without significantly affecting normalized difference vegetation indexes. Significant shifts in the taxa of the microbiome while preserving biodiversity were noted under the influence of silver nanoparticles, and the reaction of bacteria and eukaryotes was different. The eukaryotic microbiome, richer in the studied substrate, turned out to be more sensitive, showing greater qualitative and quantitative changes than the bacteriome. silver nanoparticles did not reduce the occurrence of mycorrhizal fungi, enriched the occurrence of Acidobacteriota and, with the exception of trisodium citrate reduction/sodium borohydride stabilization type, enriched the beneficial bacteria of Devosia. Within silver nanoparticles, distinct effects have been demonstrated for type with trisodium citrate reduction/sodium borohydride stabilization versus cysteamine reduction/trisodium citrate stabilization versus group: hydroxylamine hydrochloride reduction, tannic acid reduction and trisodium citrate reduction. The beneficial changes in maize rhizoplane microbiome can be attributed special to silver nanoparticles reduced using hydroxylamine hydrochloride.

Keywords: maize, soil, microbiome, silver nanoparticles

1. INTRODUCTION

According to the US National Nanotechnology Initiative's definition, "Nanotechnology is the understanding and control of matter at the nanoscale, at dimensions between approximately 1 and 100 nm, where unique phenomena enable novel applications. Matter can exhibit unusual physical, chemical, and biological properties at the nanoscale, differing in important ways from the properties of bulk materials, single atoms, and molecules. Some nanostructured materials are stronger or have different magnetic properties compared to other forms or sizes of the same material. Others are better at conducting heat or electricity. They may become more chemically reactive, reflect light better, or change color as their" (NNI, 2024). The basic and unique feature of nanoscale materials is primarily the high ratio of surface area to volume (as defined by the European Commission – above 60 m^2 cm^{-3}), which increases with decreasing particle size. This affects the reactivity, strength and adsorption properties of nanoscale materials as well as antimicrobial activity (Gorczyca *et al.,* 2021a).

Despite numerous studies in the field of nanotechnology, its assessment remains dual. Nanotechnology is a source of numerous beneficial solutions, including in agriculture (Gorczyca *et al.,* 2021a, Shao *et al.,* 2022), and simultaneously one of the important civilizational threats of the modern world (Allan *et al.,* 2021). This phenomenon can be compared to the problem of genetically modified organisms, but nanotechnology was quickly accepted by society and does not raise so many controversies (Murphy *et al.,* 2022). Agriculture has already adopted numerous nanotechnologies in key practices for sustainable intensification, such as fertilization and plant protection. Nano-fertilizers, for example: engineered metal oxide, carbon-based nano-materials, nano-coated fertilizers, nano-sized nutrients and inorganic nano-materials can reduce inputs without reducing yields and

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uptake of nutrients by plants. Nano-formulations of pesticides can be targeted and controlled release of agrochemicals for full biological effectiveness, without overdosing or leaching (An *et al.,* 2022). As Behl *et al.* (2022) wrote in a comprehensive review, nanotechnology may pose completely new threats to the environment and human health, such as modification of the nutritional value of crops, the possibility of transgenerational or trophic transport, chronic exposure on low and ultra-low concentrations, co-contamination of plants through the use of in precision farming techniques and, above all, the impact on soil biota and endobiotic organisms.

The toxicity of nanoparticles (NPs) largely depends on their physico-chemical properties, such as, among others, size, shape and chemical composition of the surface (Sukhanova *et al*., 2018). Smaller particles are more toxic due to their larger active surface area. Smaller molecules penetrate cells more easily and react with their components (nucleic acids, proteins, fatty acids, and carbohydrates). For example, pores in plant cell walls usually have a diameter in the range of 3-20 nm, which may coincide with the size of the selected NPs (Koushik *et al*., 2019). The chemical properties of NPs may be determined by the reducing agents and stabilizers used in the reaction adsorbed on the particle surface (Gorczyca *et al.*, 2021b). Stabilizers also determine the processes of oxidative dissolution of nanoparticles and thus mask or increase their toxicity (Oćwieja *et al.*, 2014). Stabilizers also enable the control of the electrokinetic properties of NPs, which results in attractive or repulsive interactions with charged living cells (Tolaymat *et al.*, 2010). Detailed knowledge about the important features of NPs is necessary to recognize their toxicity but also to compare tests carried out with different types of NPs. In this research, NPs of different size, charge and surface properties were used, which may contribute to a better understanding of the relationships.

Numerous scientific studies consistently prove that NPs are much more reactive and unpredictable than standard chemical/biological particles (Engin, 2021). Wider use of NPs in already burdened agro-ecosystems without answering the doubts related to their interaction with the components of these ecosystems may pose a serious threat. Therefore, any research related to the assessment of NPs showing whether these practices are sustainable and fully safe is necessary. Soil is the most important resources of production in agriculture. The loss of soils biodiversity and health affects the quality and quantity of yields. We are aware, confirmed by numerous scientific studies, that the value of the soil is primarily determined by its biocomponents, including microbiota currently considered one of the most important factors in the health of the soil and the plants grown in it (Vassileva *et al.,* 2022). It is indicated that the potential threats to microorganisms caused by NPs include, among others, their removal from the rhizosphere, disturbance of important biological functions, such as nitrogen fixation, phosphate solubilisation, potassium uptake, nodulation and hormone synthesis (Ameen *et al.,* 2021). NPs have been proven to damage and degrade membranes and cell walls, disrupt cellular and biochemical functions, inhibit mitochondrial and cell signalling mechanisms, and finally can cause cell apoptosis (Khanna *et al.,* 2021).

Due to the increasing global food needs, the fact that the fertility and productivity of soils is constantly deteriorating is very worrying. There is an urgent need to assess the risk of NPs – microorganisms – soil systems. Regardless of the scale of the experiment, all studies providing information on the response of soil microbiota to NPs, and consequently soil quality, are essential. This will allow metadata to be collected and clarify the biosafety of NPs, the fate of NPs in soil and their bioactivity/toxicity when released into the soil environment.

In our previous studies (Gorczyca *et al.,* 2018), we have shown that the rhizoplane microbiome depends on the plant species, and the bacterial communities found in it are sensitive to NPs to varying degrees. We determined that the surface charge of NPs under soil conditions is relevant to the microbiome. Positively charged NPs significantly reduced the amount of all bacteria in contrast to negatively charged NPs which increased their amount in the rhizoplan of Dicot plants. On the other hand, we have proven that NPs with specific surface properties can contribute to a favourable balance of phytohormones and stimulate the growth and yield of plants (Pociecha *et al.,* 2021; Matras *et al.,* 2022a). Research by other teams has also confirmed the role of NPs in increasing crop productivity by supplying plants with essential nutrients (Aqeel *et al.,* 2022) or by reducing the harmfulness of various phytopathogens (Manzoor *et al.,* 2023). On the other hand, there are many reports of adverse effects of NPs on plants at the systemic, cellular and molecular levels, including the rhizosphere (Gao *et al.,* 2023), which may pose a serious threat to crops, the environment and health of humans or animals. It has been observed that silver NPs (AgNPs) have a varied effect on macro and microorganisms and it depends on the structure and physico-chemical properties, which in turn determines the method of production of NPs (Manuja *et al.,* 2021). Most often, the toxicity of AgNPs is associated with the possibility of releasing Ag+ ions, which is also determined by the method of their production (Ferdous and Nemmar, 2020).

The aim of the study was to assess shifts in the prokaryotic and eumicrobiome of maize rhizoplanes treated with engineered AgNPs with different surface properties, produced by chemical reduction of silver nitrate(V). It was assumed that the applied method of reducing and stabilizing NPs determines the properties of the suspension and causes a variable impact on the set of microorganisms present in the rhizoplane. For comparative purposes, a solution of silver nitrate(V) was also used in the study.

2. MATERIALS AND METHODS

Silver nanoparticles (AgNPs) was prepared in the form of aqueous suspensions based on a chemical reduction process of silver ions delivered in the form of silver nitrate(V)

by selected low molar mass organic and inorganic compounds. AgNPs used in tests were labeled by the first letters of the name of compounds used during their preparation, and they were: tannic acid (TA); trisodium citrate (TC); sodium borohydride (SB); cysteamine (CH) and hydroxylamine hydrochloride (HH). Important physicochemical parameters such as hydrodynamic diameter from dynamic light scattering, polydispersity index (calculated based on DLS), electrophoretic mobility and Zeta Potential of the AgNPs used are presented in Table 1. Each type of AgNPs was quasi-spherical shape. TC and TCSB were characterized by the smallest (10 nm) and the largest size (60 nm) respectively. In the TCSB case, the synthesis was carried out using sodium borohydride as a reducing agent whereas trisodium citrate played a role of stabilizing agent (Oćwieja and Adamczyk, 2014). In turn, TC were prepared under elevated temperature and trisodium citrate played dual roles as a reducing and stabilizing agent (Pillai and Kamat, 2004). Hydroxylamine hydrochloride and tannic acid were used to obtain HH type of an average size 30 nm (Cheng *et al*., 2017), and TA of size 12 nm (Sivaraman *et al*., 2009), respectively. Both types of AgNPs were synthesized under alkaline conditions and exhibited negative surface charge similarly to TC and SBTC types. Positively charged CHSB of an average size 13 nm were prepared, reducing $AgNO_3$ by sodium borohydride in the presence of cysteamine hydrochloride (Barbasz *et al*., 2017).

Maize variety SY Talisman (Syngenta) was used in the study, which was sown in 10 L pots filled with substrate. Four replicates (pots) with 10 levelled plants after sowing 15 seeds and removed 5 divergent seedlings were used per treatment. The substrate consisted of locally collected loamy sand with a pH of 6.0; containing total nitrogen 0.2%, P_2O_5 24.8 / K_2O 20.1 / Mg 4.8 mg per 100 g of soil and commercial universal soil mixed in a volume ratio of 3:1 (v/v). Finally, the substrate was characterized of pH 6.8 and electrical conductivity (EC) of 340 μS cm^{-1}. Treatments in the phase of the first leaf of the experimental plants were done to the substrates and they were: silver nitrate(V) in concentration 10 mg L^{-1} on 1 dm^3 dry mas (DW) of soil and, respectively all types of AgNPs (TA, TC, TCSB, CHSB, HH) in the same doses. The control was untreated objects. The experiment (germination and plant growth after treatment) was carried out in a phytotron at 22°C day / 18°C night, photoperiod 16 h light / 8 h dark, light intensity 220 μmol m^{-2} s^{-1} PPFD and 60% relative air humidity. The substrate moisture level was maintained at 40-60% by watering with tap water. After treatments, plants were growing for 30 days and then assessed the normalized difference vegetation index (*NDVI*) by the PolyPen RP 410 Sheet Analyzer (Photon Systems Instruments). The *NDVI* is calculated as the difference between the reflectance of near infrared (*NIR*) and red (*RED*) divided by their sum using the formula:

$$NDVI = \frac{NIR - RED}{NIR + RED}.$$

This index allows you to determine the development status and condition of plants, and has values from –1 to 1. Higher index values correspond to higher reflection in the infrared range and lower reflection in the red range, which indicates a better condition of the plants. The dry weight (DW) of plants biomass was also assessed after drying the fresh mass of separated roots and shoots at a temperature of 80°C.

After one month of plants growth soil samples were taken to metagenomics analysis. At the beginning, the 6 g of each treatments and control soil was homogenized in ceramic mortars, and next extraction and purification were performed using GeneMATRIX Soil DNA Purification Kit (EurX, Poland) based on instruction provided with the kit. The metagenomic analysis was performed according to the following procedure: adapter ligation and sequencing of V3-V4 (bacteria) using 341F (CCTACGGGNGGCWGCAG)/805R (GACTACHVGGGTATCTAATCC) primers set and ITS (microeukaryotes) regions using ITS3F (GCATCGATGA AGAACGCAGC)/ITS4R(TCCTCCGCTTATTGATATGC) primers obtained amplicons were sequenced in Illumina MiSeq platform in 2 × 250 paired-end mode; after sequencing were performed demultiplexing and generating FASTQ files with Miseq Reporter v.2.4; data processing (+ chimera removal) using the QIIME2 methodology (Estaki *et al.*, 2020), Greengenes bacteria, UNITE fungi; metagenomic data has been deposited in an international database using the MG-RAST (Metagenomics Analysis Server) under the numbers 479 788 to 479 835.

Metagenomic data were used to calculate ecological indices: Simpson's dominance, Shannon diversity and Pielou's evenness (Simpson, 1949; Pielou, 1974; Neumann

Table 1. Selected physicochemical properties of AgNPs dispersed in the purified suspensions (pH 5.8-6.1, temperature 25°C)

AgNPs Type	Hydrodynamic diameter from Dynamic Light Scattering (DLS) (nm)	Polydispersity Index (calculated based on DLS)	Electrophoretic Mobility (μmcm Vs^{-1})	Zeta Potential (mV)
TA	12±4	0.33	–3.91±0.05	–74±1
TC	60±8	0.13	–3.68±0.11	–70±2
TCSB	10±3	0.30	–3.53±0.03	–67±1
CHSB	13±3	0.26	2.85±0.14	58±2
HH	30±6	0.20	–3.39±0.12	–64±3

and Starlinger, 2001). The obtained results were tested for normality of distribution and homogeneity of variance. ANOVA was used for parametric samples (Tukey, p=0.005) and Kruskal-Wallis test for non-parametric samples (Dunn, p=0.005). The Principal Component Analysis was performed based on the Pearson correlation coefficient. The multidimensional scaling (MDS) was performed on the basis of the Pearson correlation matrix. XLSTAT Software (Addinsoft, https://www.xlstat.com/) was used for the calculations.

3. RESULTS AND DISCUSSION

The analysis of maize plants vegetative condition (the Normalized Differential Vegetation Index) and biomass (Fig. 1) showed the differentiation of the impacts of silver nitrate and the AgNPs types used in the test. However, only in relation to the dry weight of stems and roots, the differences were significant. There was no significant differences in the normalized difference vegetation index (*NDVI*) (Fig. 1a). Maize shoots dry weight was significantly the greatest in the TA AgNPs among the treatments. The most significant reduction in growth compared to the control occurred in the silver nitrate and TC treatments (Fig. 1b). In the case of roots, all treatments significantly reduced their dry weight, including TA, compared to the control (Fig. 1c). This confirmed our previous studies, in which the AgNPs used had an adverse effect on the growth and physiological parameters of the treated monocot and dicot plants, and it was dependent on the type of AgNPs (Matras *et al.,* 2022a). In the study of Sillen *et al.* (2020), long-term maize exposure to AgNPs had not a significant negative effect on plants biomass but on the physiological state of plants, as found by the increased abundance of aquaporin and phytohormone gene transcripts, signalling an elevated stress level.

Data of metagenomic analyses of maize rhizoplane treated with silver nitrate and AgNPs are summarized in Tables 2 and 3. Calculated ecological indices of metataxa inhabiting maize rhizoplane for bacteria were characterized by low diversity. The number of reads was highest for the silver nitrate treatment and lowest for the TC treatment. For the TC, TA, CHSB and HH treatments, there was a slightly lower number of OTUs and for the silver nitrate and TCSB treatments, a slightly higher number of OTUs than in the control. Simpson's calculated dominance index was very low and uniform. No eudominant species were recorded, only dominant for an unrecognized species from Vicinamibacterales. Shannon diversity index was about 6 and Pielou evenness index was about 0.8 in each variant. Most of the recognized species were rare. The identified taxa belonged to the following Phylum (according to the numbering in Table 2): (1-4) Acidobacteriota; (5) Actinobacteriota; (6-8) Bacteroidota; (9-10) Chloroflexi; (11-12) Gemmatimonadota; (13-14) Myxococcota and (15-26) Proteobacteria. An interesting observation is, that in the rhizoplanes treated with silver compounds, Vicinamibacterales were present at the dominant and subdominant levels, which

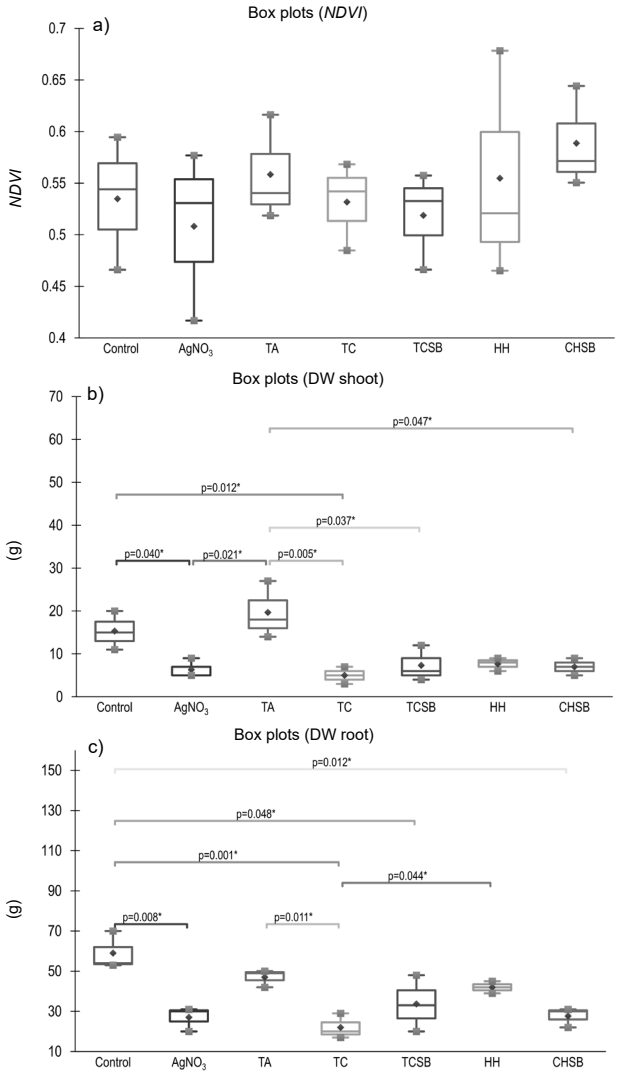

Fig. 1. Boxplots for growth parameters: a) – normalized differential vegetation index (*NDVI*), b) – dry mass of shoots, c) – dry mass of roots as effects of silver treatment on maize plants. The values in the given boxplot shown are significantly different from the control. The abbreviations on the horizontal axis indicate the type of treatment – silver nitrate and AgNPs of a given type. * significant at level alpha =0.05.

were much less numerous in the control soil. As described Huber and Overmann (2018), Acidobacteria mainly inhabit soil environments, but also some extreme habitats. In temperate soils, members of this phylum can constitute up to 70% of the bacterial community. *Vicinamibacter* have been recognized as aerobic chemoorganoheterotrophs that grow on different sugars but prefer complex proteinaceous compounds and degradation of some complex organic compounds was possible. Described *Vicinamibacter silvestris* species activity of the cysteine and valine arylaminidase and aesculin and gelatin hydrolysis. The general characteristics of Vicinamibacterales indicate on Gram-negative cells, divide by binary fission, do not form spores or capsules and are non-motile, occur either as single cells or in aggregates. Varies

Table 2. Maize rhizoplane bacteriome data: reads, OTUs, ecological indices and molecular identification

Data		Treatment						
		Control	$AgNO_3$	TA	TC	TCSB	HH	CHSB
Reads		83 291	89 592	84 522	70 785	82 742	84 497	72 936
OTUs		1 705	1 716	1 633	1 628	1 710	1 662	1 657
Simpson's dominance		0.005	0.005	0.006	0.005	0.005	0.006	0.004
Shannon diversity		6.031	6.042	5.934	6.003	6.040	5.931	6.046
Pielou's evenness		0.811	0.811	0.802	0.812	0.811	0.799	0.816
Taxon identification								
No	Sample ID							
1	B_OTU_01_f	0.00	4.32	5.54	5.51	4.95	5.48	4.35
2	Vicinamibacteraceae	2.34	2.25	1.38	1.58	2.09	1.52	2.29
3	Subgroup_17	1.24	1.37	0.86	0.90	1.12	0.97	1.13
4	*Bryobacter*	1.01	0.84	1.29	1.23	0.97	1.14	1.04
5	IMCC26256	1.03	0.92	0.98	0.93	0.93	0.95	0.95
6	B_OTU_02_g	1.94	1.74	2.07	1.63	2.13	1.95	1.98
7	B_OTU_03_g	1.23	0.62	2.06	1.62	1.34	2.12	0.86
8	*Terrimonas*	1.13	1.10	0.92	0.84	1.09	0.94	1.20
9	KD4-96	2.54	3.07	2.46	2.69	2.59	2.81	2.32
10	JG30-KF-CM66	1.08	1.29	0.90	1.00	1.09	0.89	0.95
11	B_OTU_05_g	3.00	3.31	3.21	3.01	3.27	3.16	3.30
12	*Gemmatimonas*	0.63	0.50	1.00	0.81	0.68	0.84	0.75
13	BIrii41	1.69	1.36	2.08	2.00	1.75	2.40	2.25
14	*Haliangium*	1.39	1.10	1.47	1.50	1.39	1.74	1.50
15	SWB02	1.28	1.42	1.12	1.20	1.42	1.14	1.44
16	B_OTU_06_g	1.07	0.74	1.93	1.73	1.18	1.77	1.00
17	*Micropepsis*	0.23	0.17	1.00	0.46	0.33	0.49	0.26
18	*Devosia*	2.18	1.70	2.24	2.19	2.01	2.57	2.19
19	B_OTU_06_g	1.60	1.83	1.45	1.68	1.58	1.43	1.61
20	*Pseudolabrys*	0.83	0.80	1.43	1.42	0.97	1.38	0.75
21	*Bauldia*	1.16	1.17	1.39	1.29	1.21	1.34	1.08
22	B_OTU_07_g	0.96	0.94	0.67	0.84	0.88	0.77	1.04
23	B_OTU_08_o	1.06	1.27	0.97	1.00	1.25	0.97	1.19
24	*Acidibacter*	1.74	1.41	1.09	1.06	1.23	1.22	1.75
25	*Luteimonas*	0.87	0.69	1.03	0.79	0.78	0.86	0.76
26	*Rhodanobacter*	0.56	0.47	1.33	0.94	0.59	1.00	0.41
Domination level		Eudominant	Dominant	Subdominant		Rare	Occasional	
		>10%	5.01-10%	2.01-5%		1.01-2%	<1%	

catalase and cytochrome c oxidase activity have been detected for them. As Huber and Overmann (2018) reported that Acidobacteria are highly abundant in non-acidic soils and are highly diverse. Due to the lack of clear data, it is difficult to diagnose the benefits in the occurring community shifts under the influence of the applied AgNPs.

The largest amount (12) of OTUs was found in Proteobacteria, including the Alphaproteobacteria class, where *Devosia* was a subdominant in all variants, only in the case of silver nitrate it was rare. *Devosia* comprises a group of motile, gram-negative bacteria. The first recognized species of the genus was *Devosia riboflavina* (formerly *Pseudomonas riboflavina*) described by Foster (1944) from riboflavin-rich soil. Numerous members of this genus still are reported from diverse ecological niches. Although *Devosia* distribution is ubiquitous including their presence in nodules of legume plants, they have been mainly reported from contaminated soils with for example

Table 3. Maize rhizoplane microeukaryotes data: reads, OTUs, ecological indices and molecular identification

Data		Treatment						
		Control	$AgNO_3$	TA	TC	TCSB	HH	CHSB
Reads		51 516	36 796	70 386	73 365	50 714	68 430	61 092
OTUs		460	432	413	468	476	433	465
Simpson's dominance		0.051	0.093	0.057	0.063	0.049	0.073	0.037
Shannon diversity		3.851	3.670	3.726	3.719	3.890	3.553	4.064
Pielou's evenness		0.628	0.604	0.618	0.604	0.631	0.585	0.661
Taxon identification								
No	Sample ID							
1	Penicillium	1.16	0.72	0.74	0.65	0.83	0.52	1.20
2	Byssochlamys	2.05	1.01	1.61	1.56	1.63	1.83	1.80
3	Chrysosporium	2.48	1.84	8.71	4.00	3.90	5.91	2.17
4	Oidiodendron	0.98	0.79	0.34	0.40	0.86	0.29	1.22
5	F_OTU_01_o	0.27	0.17	0.58	0.21	0.19	0.11	1.38
6	Pseudogymnoascus	0.77	0.88	3.50	0.74	1.22	0.83	0.80
7	Ascobolus	0.20	0.87	0.03	0.01	0.34	0.06	1.00
8	Terfezia	0.03	1.29	0.01	0.02	0.20	0.03	3.13
9	F_OTU_02_f	2.61	0.59	1.28	2.32	0.87	2.86	0.81
10	F_OTU_03_o	0.89	0.72	1.36	0.76	0.63	1.14	0.54
11	Candida	0.16	1.10	0.02	1.84	1.10	1.25	1.49
12	Blastobotrys	0.51	0.57	0.19	0.31	0.92	0.24	1.08
13	Schwanniomyces	0.70	0.49	0.24	0.29	0.63	0.42	1.00
14	Fusarium	0.03	0.78	0.52	1.75	0.37	1.00	0.38
15	Chaetomium	1.72	1.14	0.39	1.07	2.10	0.36	2.70
16	Humicola	0.71	0.58	1.56	0.37	0.86	0.43	0.73
17	Apodus	0.01	0.00	0.02	0.01	0.00	0.01	0.02
18	F_OTU_04_f	0.35	0.32	0.07	0.15	0.24	0.03	1.26
19	F_OTU_05_o	0.82	1.07	0.78	1.07	2.22	0.88	2.58
20	F_OTU_06_o	1.01	0.11	0.04	0.09	0.07	0.07	0.10
21	F_OTU_07_p	1.17	2.08	1.28	0.34	2.15	0.27	8.97
22	Entoloma	12.26	1.86	8.42	11.15	10.34	12.47	7.48
23	Mucronella	1.05	1.07	1.90	2.06	1.52	2.36	0.94
24	Xerocomellus	0.17	0.42	1.01	0.36	0.18	0.44	0.28
25	F_OTU_08_f	0.00	1.22	0.02	0.00	0.03	0.00	0.00
26	F_OTU_09_o	8.30	3.40	15.87	18.49	7.26	19.80	10.04
27	Mortierella	0.97	1.28	1.28	0.86	1.28	0.83	1.65
28	F_OTU_10_k	5.60	26.47	3.80	5.85	13.51	3.28	4.31
29	Thaumatomonas	4.80	2.55	2.34	2.70	1.79	2.50	0.89
30	F_OTU_11_p	15.70	12.94	10.27	9.97	10.18	9.64	8.02
31	Apoikiospumella	0.76	1.06	1.82	2.25	1.16	2.46	0.74
32	Spumella	1.18	1.95	1.38	1.67	1.18	1.24	1.16
33	Mallomonas	1.14	0.97	0.32	0.69	0.99	0.50	1.42
34	Halteria	0.00	0.38	0.00	0.40	1.00	0.37	0.47
35	Anteholosticha	0.00	0.46	0.00	1.57	1.14	0.74	1.04
36	F_OTU_12_p	0.31	1.05	0.30	0.83	1.84	1.02	0.81
37	Rhodomonas	6.15	2.63	5.98	2.87	3.17	4.89	1.61
38	Cyanophora	0.86	0.73	0.92	1.20	0.48	0.97	0.28
39	Glaucocystis	1.01	2.11	0.79	0.87	0.68	0.49	1.28
Domination level		Eudominant	Dominant	Subdominant		Rare		Occasional
		>10%	5.01-10%	2.01-5%		1.01-2%		<1%

mycotoxins and hydrocarbon pesticides. *Devosia* became yet well known for their dominance in soil habitats and beneficial role *e.g.* nitrogen fixation and bioremediation potential. Genome-scale positive evolution analysis of the studied *Devosia* species highlighted genes related to growth, detoxification, chemotaxis and stress response. The study will

highlight the plasticity of the *Devosia* spp. genome, ensuring adaptation, bioremediation and high potential for the use of a wide range of substrates. Strong utilization of toxins by *Devosia* highlights their future applications in bioremediation (Talwar *et al*., 2020). The remaining subdominant OTUs from Proteobacteria could not be assigned to a genus.

The number and diversity of microeukaryotes was characterized by much greater variability (Table 3). The number of reads was highest for the TC and TA treatments (above 70 000), with 51 516 in the control. A significant decrease in reads occurred in the silver nitrate treatment (only 36 796). The number of OTUs of microeukaryotes was equal and ranged from 413 to 476. Moderate differences in the dominance index were noted – the highest (0.093) for the silver nitrate treatment and the lowest (0.037) for the CHSB treatment. For CHSB treatment the highest diversity index was also shown. Pielou evenness index in all variants was balanced in the range from 0.585 (HH) to 0.631 (TCSB).

The isolated microeukaryotes belonged to the identified Phylum (according to the numbering in Table 3): (1-21) Ascomycota; (22-26) Basidiomycota; (27) Mortierellomycota; (29-30) Cercozoa; (31-33) Ochrophyta; (34-36) Ciliophora; (37) Cryptophyta and (38-39) Glaucocystophyta. The highest number of OTUs was found for Ascomycota (21 OTUs), but there were no eudominants. There were 5 OTUs within the Basidiomycota, including 2 eudominants, and one of them, the mycorrhizal fungus *Entoloma*, was eudominant in the control and the TC, TCSB and HH treatments and significantly reduced to rare in the silver nitrate treatment. Treatment with AgNPs (excluding TCSB) had a much more favorable effect on the occurrence of another, unspecified to the genus, beneficial root endophyte belonging to *Sebacinales*, which from the dominant position in the control reached the position of eudominant in the treated soil, and again only silver nitrate reduced the occurrence of this beneficial genus. Among the microeukaryotes not classified as fungi, the following were found in maize rhizoplane: Cercozoa and Ciliophora bacterivores, golden algae Ochrophyta and red algae Cryptophyta and Glaucocystophyta. It can be assumed that the presence of these microeukaryotes was related to the irrigation of substrates, since they are aquatic organisms, but this need future careful recognition. The effect of the applied treatments with silver compounds on these groups was not unambiguous. AgNPs, similarly to silver nitrate, caused an increase or decrease in the number of these microeukaryotes, depending on their type – Cercozoa eudominating bacterivores for this group were more strongly reduced by AgNPs than by silver nitrate. The effect on gold algae was inconclusive – Chrysophyceae were stimulated by all treatments except CHSB, and Synurophyceae were the opposite. The silver compounds used significantly reduced the occurrence of Cryptophyta red algae, which dominated the control.

Principal component analysis (PCA) and multidimensional scaling (MDS) (Fig. 2) showed a clear disaggregation of the silver compounds used in relation to their effect on the growth of young maize plants and shifts in the rhizoplane microbiota. Silver nitrate showed a significantly different effect from AgNPs. According to Bakr *et al*. (2022), there are many reasons why silver nitrate may be more or differently toxic than AgNPs. Firstly, the solubility of silver nitrate is much higher than that of AgNPs. Moreover, in a molar ratio, the concentration of silver nitrate ions is higher than the concentration of ions from AgNPs of the same mass, and the degree of elimination of AgNPs is higher than that of silver nitrate. The effect in metagenomic analysis observed in these studies may be partially due to the slower release of ions from AgNPs (outside or inside cells) compared to silver nitrate, but the toxicity reflected in the lower number of reads concerned eukaryotic microorganisms but not bacteriomes. This may be explained by a different mechanism of oxidative stress, as proven by Ribeiro *et al*. (2015) for eukaryotes treated with silver nitrate and AgNPs or the function of soil bioligands influencing the ratio of dissolution rate to uptake. Numerous researches (Ghobashy *et al*., 2021) show that the degree of toxicity caused by the soluble ion fraction and the particulate fraction of the tested silver compounds is unclear and probably varies depending on the experimental conditions.

Within the AgNPs forms, in our research, distinct effects have been demonstrated for (1) TCSB; (2) CHSB and (3) similar for HH, TA, TC. This did not confirm the conclusion, which is common in other studies, that the size of AgNPs affects the toxicity to microorganisms (Kong *et al.,* 2020) because the TCSB, CHSB and TA used in this study had similar dimensions (10-13 nm) and are significantly smaller than TC (60 nm) and HH (30 nm) (Table 1). The significant effect of the surface charge of AgNPs was partially confirmed, because CHSB with a positive surface charge had a different effect on the analysed parameters than other negatively charged AgNPs (Table 1). The obtained results confirm our previous research (Matras *et al.,* 2022b) that the surface properties of AgNPs resulting from the production method (chemical compounds used in the reduction and stabilization of NPs) play an important role in the impact of AgNPs released into the environment. Chemical compounds of reducers and reaction stabilizers adsorbed on the surface layer may determine the properties of AgNPs (Oćwieja *et al.,* 2014). This may determine the release of ions, aggregation, biostatic and spectral properties (Akter *et al.,* 2017) as well as the formation of a protein crown on the biointerface, which may determine their toxicity (Durán *at al.,* 2015). The performed studies confirmed earlier reports that the reaction of organisms (such as plants, soil microorganisms) to AgNPs treatment depends on many factors, and one of the important ones is the surface properties of AgNPs resulting from the method of their production.

Taking into account all the obtained results and sum up, it should be stated that hormesis, already reported in numerous publications as an adaptive response of biological systems to moderate environmental challenges

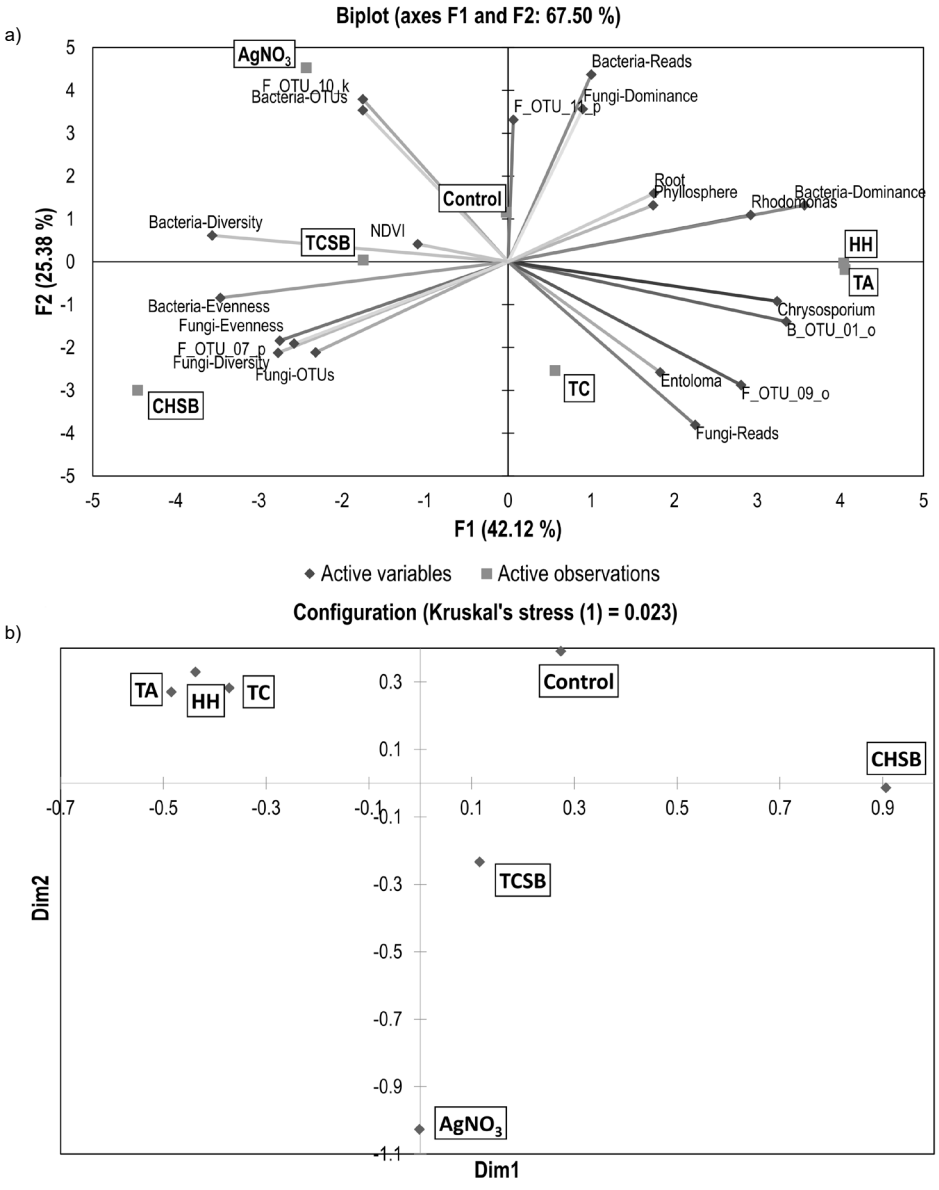

Fig. 2. Analysis of the: a) principal components PCA and b) multidimensional scaling MDS of experimental data collected. TA, TC, TCSB, CHSB and HH means given type of AgNPs.

(Calabrese and Baldwin, 2000; Iavicoli *et al.*, 2018; Sillen *et al.*, 2020; Juárez-Maldonado *et al.*, 2021) was probably the cause of favourable changes in shifts of maize rhizoplane microbiome. The hormesis was noted mostly in the case of HH AgNPs type where beneficial *Devosia* and *Entoloma* were more numerous than in the control. In a study similar to ours, Sillen *et al.* (2020) using commercial uncoated AgNPs with variety of sizes due to clustering of the original particles of ca. 20 nm in higher concentration *i.e.* 100 mg kg^{-1}, observed the effect of hormesis on maize plants and the lack of significant, beneficial shifts in the microbiota of its rhizoplanes. It can be assumed that the hormesis of plants and microbiota takes place in different concentrations of AgNPs, and for microorganisms the concentration should be lower than for plants. This requires a detailed assessment, also taking into account the type of AgNPs used. Our research confirmed that the important features in the interaction between biota and AgNPs are surface properties of particles.

4. CONCLUSIONS

Soil treatment of maize in the initial phases of growth with silver nitrate(V) and silver nanoparticles at concentration of 10 mg·L^{-1} reduces the accumulation of dry matter, especially roots, but does not show a negative effect on the Normalized Difference Vegetation Index.

The rhizoplane microbiome of maize treated with silver nitrate(V) and silver nanoparticles shows shifts depending on the type of silver compound and microbiota taxa.

Silver nitrate(V) shows significantly different effects on the rhizoplane microbiome than silver nanoparticles, which was shown especially for mycorrhizal fungi identified in the rhizoplane and beneficial bacteria *Devosia* genus.

The effects of silver nanoparticles are not dependent on the particle size but on the surface properties of the stabilizing layer, including the surface charge.

The release of silver nanoparticles into the environment may disturb soil homeostasis but in low concentration can also cause hormesis effect in beneficial microorganisms depended on silver nanoparticles surface properties.

Conflicts of Interest: The Authors declare they have no conflict of interest.

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