



Toxicity of Silver Nanoparticles and Their Removal Applying Phytoremediation System to Water Environment: An Overview

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Abstract

The widespread use of silver nanomaterials potentially harms the health of the whole ecosystem, especially the aquatic environment. Silver nanoparticles (AgNPs) are directly released into environment from washing machines, colloidal silver medicines, and other AgNPs-containing products. Review of ecotoxicological studies and the prediction of the future environmental concentrations (PEC) shows the presence of a toxic level of AgNPs in the surface water and reveals their effect and risks to aquatic organisms. However, the AgNPs transport behavior, their transformation in the natural environment, and how this behavior poses a risk to human and ecosystem health are significant issues that have not been clearly known; thus, there is a pressing need to investigate them and provide effective solutions. This study reviews the potential of macrophytes to remove AgNPs in aqueous solutions. It also discusses the impact of AgNPs on water environments, their toxicity to aquatic organisms, and the phytoremediation functions.

Keywords: Silver nanoparticle; Macrophyte; Phytoremediation; Surface water

1 Introduction

In recent years, manufactured nanoparticles (NPs) and nanotechnology have grown very quickly and have been broadly used. Annually, around 500 tons of silver nanoparticles (AgNPs) are produced, representing a major class of engineered NPs frequently used in manufactured products and also a significant potential for environmental impact [1, 2, 3, 4, 5, 6]. AgNPs have widely used in different industries, medical imaging, textile, house products, etc. because of not only their high antibacterial properties, but also high electrical and thermal conductivity [2, 5, 7, 8, 9, 10].

However, these AgNPs containing products mostly end up in the environment and aquatic systems due to accidentally leakage during the manufacturing, production, distribution and disorderly disposal. These concerns have led to several studies investigating the detection of the particles in the aquatic environment, the fate and transformation of AgNPs in the aquatic environment, and the potential environmental toxicology of these AgNPs to aquatic organisms [11, 12]. Literature shows the extensive use of AgNPs has exerted adverse effects on the aquatic ecosystem, especially

bacteria, plants, fishes, etc. and, thereby indirectly to humans [13, 14, 15, 16]. Thus, there is a crucial need for further research aiming at understanding the environmental behavior of NPs and predicting their environmental implications [4, 10].

The AgNPs-contaminated aquatic system is a possible route for human exposure when it is used as a source of potable water. Different people show different reactions to this exposure such as rash, inflammation, swelling, and mild allergic reaction, and in some cases, no effect is observed. Living organisms (e.g., microbes involving in nutrient cycle, waste composting, and other beneficial and harmful bacteria) in the aquatic environment can be also affected directly. Previously-conducted studies on toxicology have proved that AgNPs can cause DNA damage, chromosomal aberrations, and even a slight effect on the brain as revealed by tests on rats, and also their effect on the early development of zebrafish embryos has been confirmed. In addition, there are some biological concerns due to the fact that harmful microbes might become resistant toward AgNPs that are used as anti-microbe agents and also for coating some medical equipment.

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2 Toxicity of Silver Nanoparticle

The toxicity of AgNPs and the dynamic nature of these particles in complex media and living systems have been investigated for many years; however, the risk toward human health, particularly the mammalian cells is still unclear [17, 18, 19].

Table 1: The Toxicity of AgNPs

No	Studies	Effects of the AgNPs Toxicity	References
1	Fish: Japanese medaka	Decreased hatchability of embryos and eye size	[25]
2	Biological systems: virus, bacteria, fungi, Protist, animal cell lines, and human cancer cell lines	Inhibitory effect on all biological systems tested	[26]
3	Fish: Zebrafish	Inhibited hatchability behavioral retardation in early-life stages; Effects on embryos; Neurodevelopmental toxicity altered locomotion	[27, 28]
4	Human glioblastoma cells (U251) human lung fibroblast cells (IMR-90)	Reduced ATP content of the cell; Inactivate enzymes from several cellular pathways depolarizing and mitochondrial membrane; Damaging lysosomes; Increased the production of reactive oxygen species (ROS) in a dose-dependent manner	[29, 30, 31, 32]
5	Mammal: Mice	Cross the blood-brain barrier and caused long-term contextual memory impairment	[33]
6	Sea urchin	Development of abnormalities	[4]
7	Ecotoxicological (ng L^{-1})	Affecting prokaryotes, invertebrates, and the fish	[5]
8	Plant: Zucchini seeds	Reduced the zucchini biomass and transpiration rate	[34]
9	Microbial profile of caecum	Affecting population size of certain types of bacteria	[35]
10	Roach (<i>Rutilus rutilus</i>) and Goldfish (<i>Carassius auratus</i>)	Symptom of poison with Cd: anxiety, color vision, increased mucus secretion, and death with the open mouth	[36]
11	Gram-positive and Gram-negative bacteria	No bactericidal activity in concentration of AgNPs up to 128 $\mu\text{g/ml}$	[37]

In the aquatic environment, many factors that affect the fate and transformation can be effective on the toxicity and characteristics of AgNPs. Many parameters, including time, influence the presence of AgNPs in water environment, which might be still in nanoparticle shape, suspension in surface water, aggregate/agglomerate, dissolved, or bound with natural organic materials or other substances that present in the aquatic system. Dissolution also may occur when oxidation happens, which changes Ag⁰ to Ag⁺¹. This ionic state of Ag is more toxic toward aquatic organism; however, this oxidation process is complicated because it depends on time and water physicochemical characteristics [2, 20, 21, 22, 23, 24]. A number of important studies carried out into the toxicity of AgNPs are listed in Table 1.

3 Phytoremediation in Aquatic Media

Phytoremediation is a natural process through which plants are used to remove contaminants from soil, air, and water resources. Phytoremediation has a few types: accumulation, degradation, stabilization, rhizodegradation, rhizofiltration, and volatilization of the contaminant in the environment [38, 39, 40]. Generally, for water or aquatic environment remediation, macrophytes are used as phytofiltration or rhizofiltration, which involves the removal of contaminants by adsorption/absorption through the plant roots. Macrophytes also do the task of phytoaccumulation through accumulating contaminants in their biomass such as tissues of the roots, stems, or leaves. This process can reduce pollutants in water environments such as wetlands and estuary areas. Phytoremediation is a useful technique for removing pollutant from the contaminated water. These macrophytes can go through phytomining process that refers to harvesting back the metals from water environment for recycling purposes [41].

The macrophytes used for rhizofiltration of AgNPs require an extensive root system capable of tolerating the contaminated water source and aggressively accumulating and filtering the contaminants. Moreover, macrophytes are better than invasive species that quickly reproduce for self-sustaining [42, 43, 44].

The phytoremediation of contaminated water can be normally performed with a low cost since no expensive equipment and chemical is needed for this process. The techniques of rhizofiltration and phytoaccumulation can improve water and soil quality with only a negligible site disruption [38, 40]. However, the appropriate use of macrophytes needs more experiments, analyses, and observations since AgNPs can negatively affect the plants itself. Once the AgNPs enter the aquatic system, the unclear fate and transformation of AgNPs will affect the mechanism of this phytoremediation. AgNPs can be transformed physically and chemically by aggregation or disaggregation, bonding with natural organic matter, or dissolution, which affect the stability of AgNPs, the uptake and accumulation of AgNPs by macrophytes, their bioavailability as well as their toxicology effect toward living organisms and phytoremediator [4, 9, 10, 45, 46, 47]. The toxicity of AgNPs prevents macrophyte from effectively remediating the contaminated water. Microorganisms and bacteria communities that surround and are attached to rhizosphere are supposedly very beneficial for phytoremediation potential; however, the toxicity of AgNPs is harmful to these microorganism communities and affect their functioning in their

ecosystems [46, 48].

4 The Uptake of Silver Nanoparticles in Aqueous Solution

Plants are key assets for a successful phytoremediation. The suitable plants must be able to clean the contaminants, especially metal nanoparticles in water sources. Several phytoremediation studies have been done to examine the capabilities of macrophytes in filtrating the AgNPs-contaminated water and wastewater. Usually, a phytoremediation study is conducted in lab to get enough data before running for the pilot scale and real pond or river. Different plants and methods have been investigated to imitate and understand the natural process of phytoremediation. Mostly all the plants used in this study are collected from actual ponds or rivers; then, they are quarantined in artificial ponds/aquariums before being used for further experiments. In addition, some plants are grown in a greenhouse.

Then, to run the experiment, plants of the same size are chosen and washed thoroughly by tap water and distilled de-ionized water (DDIW) to clean from any impurities. Some other researchers just collect the similar size of plants from the river or actual pond and then wash them with deionized water before keeping in a nutrient solution. The experiments are normally performed with three to five replicates. The media sample is taken followed by a scheduled time and the visual changes of the plants are checked to see whether there is any toxicity effect of silver on the plants.

At the end of the experiment, all the plants are taken out of the media and are examined. The plants are again washed thoroughly, then are divided into roots, stem, and leaves before air dry at room temperature or by oven dry. All the plants parts are then ground to get fine powder for further analyses.

Table 2: Summary of Research Conducted into AgNPs Removal

Plants	Pollutants	Methodology	Results (Recovery/uptake/absorb/ removal)	Ref.
<i>Pistia stratiotes</i> (water lettuce)	AgNPs, Ag ⁺ (reduction of silver nitrate by sodium borohydride)	0.02, 0.2, and 2 mg L ⁻¹ 48 hours with a light-dark cycle of 14-10 h	Recovery of 96% , 54% and 20% for 0.02, 0.2, 2 mgL ⁻¹ of AgNPs respectively	[52]
<i>Phragmites australis</i> (salt marsh plant)	AgNPs Ag ⁺	10mg L ⁻¹ , natural at 7 days	Metal level in plants is below the LOQ (2 µg gdry plant -1).	[49]
<i>Pleuston-Limnobia laevigatum</i> , <i>Pistia stratiotes</i> L., and <i>Salvinia natans</i> (L.) All, <i>Elodea canadensis</i> Michx., <i>Najas guadelupensis</i> (Spreng.) Magnus, <i>Vallisneria spiralis</i> L., and <i>Riccia fluitans</i> L.	The mixture of colloidal solutions of metal nanoparticles (Mn, Cu, Zn, Ag + Ag ₂ O)	Mn : 0.75 mg L ⁻¹ Cu : 0.37 mg L ⁻¹ Zn : 0.44 mg L ⁻¹ Ag + Ag ₂ O : 0.75 mg L ⁻¹ At 7 days for each pollutant	Uptake of Ag : P. stratiotes and S. natans (76%) and L. laevigatum and E. canadensis (71%). R. fluitans (65%) V. spiralis (59%).	[50]
<i>Egeria densa</i> / <i>Elodea densa</i> (waterweed)	Ag ⁺ and AgNps (reduction of AgNO ₃ by humic acids)	5, 10, 20, 30, 50, and 100 ppm Ag ⁺ in MHRFW (used Silver nitrate). 5, 10, 20, and 30 ppm AgNps. At 7 days	Total silver absorbed increases by increasing concentration of AgNPs	[51]
<i>Pistia stratiotes</i> (water lettuce) <i>Eichornia crassipes</i> (water hyacinth)	AgNPs (natural reducing agent, Muntingia calabura sp. Leaves)	7 – 10 ppb for 96 hours	Removal of AgNPs for both plants were 61%	This study

The control plants are also subjected to the same procedure to assess Ag initial levels in the selected plants. For determination of the levels of Ag and the changes of total AgNPs in plants and media, samples are subjected first to microwave digestion before being analyzed by atomic absorption spectrophotometry (AAS) or ICPMS (depending on the given experiment), and FEG ESEM (FEI) is also used to examine the plants [45, 49, 50, 51, 52, 53, 54]. Many types of methods have been proposed in literature to identify the plants that can be better used for phytoremediation of AgNPs and silver ion. Each method is designed in a way to accomplish the objective defined for that specific study. Table 2 summarizes the studies carried out to remove AgNPs from water.

5 Plants Mechanism

The results of some studies have shown that aquatic plants have the capability of removing AgNPs at a rate of around 30-100%. This proves that aquatic plants or macrophytes are useful in green cleaning technology.

In [42, 43, 49], macrophytes of *Eichornia crassipes* (water hyacinth) and *Pistia stratiotes* (water lettuce) were used for phytoremediation of AgNPs in aqueous solution. The studies were aimed to investigate the uptake of AgNPs by water hyacinth and water lettuce in controlled conditions and evaluate the effect of AgNPs on plants health. These macrophytes were selected and investigated as these two plants are known to be highly capable of tolerating metals and aggressively accumulating contaminants and nutrients. Moreover, they have extensive root systems, are known as invasive species of floating macrophytes, and have already shown promising results for heavy metal water purification purposes.

Green technology is used to utilize plants for the extraction of certain elements depending on their properties and capabilities. In this regard, different plants show different results. The present study was conducted on the removal of AgNPs using water lettuce and water hyacinth. The obtained results are shown in Figures 1 and 2.

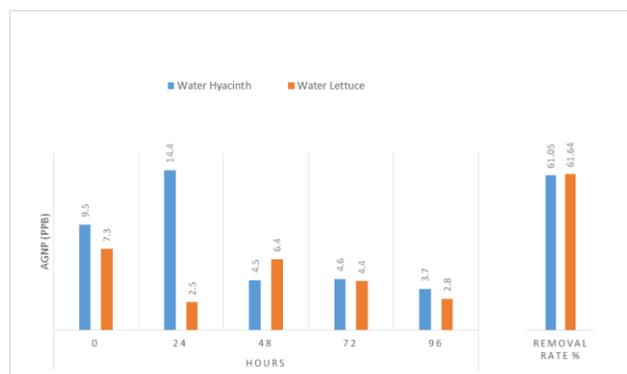


Figure 1: Removal of silver nanoparticle in aqueous solution for 96 hours and the removal rate of the silver nanoparticle

Findings showed that both the above-mentioned plants can survive in lower concentrations ($7.0\text{-}10.0\ \mu\text{gL}^{-1}$) of AgNPs. In this condition, contaminants are retained within the plant. This low concentration does not exert any physical effect on the plants. The physical form of the plants remained the same until the end of the

experiment. However, the result might be different in a higher concentration of AgNPs.

The results of the previous study showed that *P. stratiotes* can reduce the concentration of AgNPs and ions in aqueous solution. The plants also could survive under $0.02\ \text{ppm}$ of AgNPs; however, the increase of the AgNPs concentration to $2\ \text{ppm}$ caused the plant's physical shape to be degraded over time; the leaves were wilting, discolored, and browned as the root detached from the aerial part of the plants [49]. While, the results of a study conducted by Leonard Bernas [51] on phytoremediation of silver using waterweed showed that the toxicity of silver can be seen by a deterioration in the health of plants when exposed to silver in concentrations as low as $5\ \text{ppm}$. Plant health is generally interrupted at concentrations of $20\ \text{ppm}$ Ag^+ and AgNPs, as revealed by the significant browning of the leaves. Higher concentrations of silver had a more pronounced effect on the plants for all measurements. The *E. densa* tests with Ag^+ at 50 and $100\ \text{ppm}$ caused the leaves to turn brown and undergo chlorosis (insufficient chlorophyll production by leaves) and necrosis (cell death from toxic environments and extracellular factors).

Therefore, the presence of Ag^+ or AgNPs causes an obvious decline in plant health over the exposure period. The obtained results of present study show that water lettuce and water hyacinth have almost the same percentage removal rate of 61% . This means that the plants have almost equal ability in removing AgNPs at low concentrations. While, Olkhovych [50] showed that the *P. stratiotes* and *S. natans* can remove all studied metal nanoparticles from water; however, *N. guadelupensis* was found more preferable for phytoremediation of AgNPs with the removal rate of 82% .

All these plants can be used for phytoremediation and have the capability to accumulate Ag of water resources. Results obtained from the present study investigating the accumulation or uptake of silver by water lettuce and water hyacinth are presented in Figure 2.

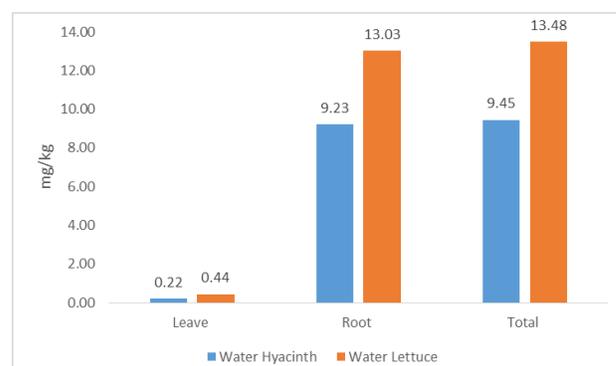


Figure 2: The accumulation of silver nanoparticle in leaves and roots of water hyacinth and water lettuce

The analysis showed that the root parts of both plants play a significant role in the accumulation of AgNPs compared to their aerial parts. Another study [49] used salt marsh plant as phytoremediator, and the result showed that the accumulation was only detected in roots and rhizomes. Any Ag was not detected in the aerial part of the plant. However, this plant can accumulate AgNPs only in the absence of rhizosediment. Kadukova [55]

claimed that metal in water can enter the plants by the intracellular (symplastic) and extracellular (apoplastic) pathways. However, the cell wall of plants limits the extracellular transport in the root and lead to lower detection of Ag in the aerial part of plants. For this experiment, only the root parts of the plant had to submerge in the media; thus, the majority of the accumulated metal nanoparticles are more likely to be attached and move in through the plant roots. Therefore, these particles have low interaction with the aerial part of the plant since the cell wall has a high cation exchange capacity. However, silver is known to be toxic to some plants, inhibiting enzymes and altering the permeability of the cell membrane wall. Thus, a number of silver species are still able to make their way into the leaves of plants. This indicates that silver is not necessarily bound only to the root tissue of plants. The silver species can be transported through damaged cells or these species can utilize transport proteins to translocate from roots to leaves [56, 57, 58].

5 Conclusion

AgNPs rapidly change in particle size and surface chemistry upon exposure to media and interaction with their chemical environments such as salinity and pH. These exposures need to be considered in evaluating the hazard and risks of AgNPs toward the aquatic ecosystem since they affect the speciation of AgNPs and their toxicity. Toxic effects can be enhanced or decreased due to the transformations of AgNPs in the water environment by bioaccumulation and the dissolution of AgNPs to the formation of Ag⁺ ions. On the other hand, phytoremediation is a natural method that utilizes the plant's metabolic system to remove, reduce, degrade, assimilate, and metabolize AgNPs in water sources and store them in the biomass of the plants. The appropriate selection of aquatic plants can remediate AgNPs-contaminated water. Heavy metal contamination is a growing environmental concern; thus, successful removal using phytoremediation approaches with duckweeds, waterweeds, floating, and submerge macrophytes should be studied further to provide an environmentally benign solution to the problem. Additional studies need to be performed using macrophytes and similar organisms to determine the most effective way for silver species phytoremediation.

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Ethical issue

Authors are aware of, and comply with, best practice in publication ethics specifically with regard to authorship (avoidance of guest authorship), dual submission, manipulation of figures, competing interests and compliance with policies on research ethics. Authors adhere to publication requirements that submitted work is original and has not been published elsewhere in any language.

Competing interests

The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

Authors' contribution

All authors of this study have a complete contribution for data collection, data analyses and manuscript writing.

References

1. Ellis, L.-J. A., Baalousha, M., Valsami-Jones, E., Lead, J. R. Seasonal variability of natural water chemistry affects the fate and behaviour of silver nanoparticles. *Chemosphere*. 2018. 191, 616–625.
2. Zhang, C., Hu, Z., Deng, B. Silver nanoparticles in aquatic environments: Physicochemical behavior and antimicrobial mechanisms. *Water Research*. 2016. 88, 403–427.
3. Lodeiro, P., Achterberg, E. P., Pampín, J., Affatati, A., El-Shahawi, M. S. Silver nanoparticles coated with natural polysaccharides as models to study AgNP aggregation kinetics using UV-Visible spectrophotometry upon discharge in complex environments. *Science of the Total Environment*. 2016. 539, 7–16.
4. Buric, P., Jaksic, Z., Stajner, L., Sikiric, M.D., Jurasin, D., Cascio, C., Calzolari, L., Lyons, D.M. Effect of silver nanoparticles on Mediterranean Sea urchin embryonal development is species-specific and depends on the moment of first exposure. *Marine Environmental Research*. 2015. 111, 50-59.
5. Fabrega, J., Zhang, R., Renshaw, J.C., Liu, W.T., Lead, J.R. Impact of silver nanoparticles on natural marine biofilm bacteria. *Chemosphere*. 2011a. 85, 961-966.
6. Fabrega, J., Luoma, S.N., Tyler, C.R., Galloway, T.S., Lead, J.R. Silver nanoparticles: behavior and effects in the aquatic environment. *Environment International*. 2011b. 37, 517-531.
7. Kumahor, S.K., Hron, P., Metreveli, G., Schaumann, G.E., Vogel, H.J. Transport of citrate-coated silver nanoparticles in unsaturated sand. *Science of the Total Environment*. 2015. 535, 113-121.
8. Braun, A., Klumpp, E., Azzam, R., Neukum, C. Transport and deposition of stabilized engineered silver nanoparticles in water-saturated loamy sand and silty loam. *Science of the Total Environment*. 2015. 535, 102-112.
9. Metreveli, G., Philippe, A., Schaumann, G.E. Disaggregation of silver nanoparticle homoaggregates in a river water matrix. *Science of the Total Environment*. 2015. 535, 35-44.
10. Gambardella, C., Costa, E., Piazza, V., Fabbrocini, A., Magi, E., Faimali, M., Garaventa, F. Effect of silver nanoparticles on marine organisms belonging to different trophic levels. *Marine Environmental Research*. 2015. 111, 41-49.
11. McGillicuddy, E., Murray, I., Kavanagh, S., Morrison, L., Fogarty, A., Cormican, M., Dockery, P., M. Prendergast, N. R., Morris, D. Silver nanoparticles in the environment: Sources, detection, and ecotoxicology. *Science of the Total Environment*. 2017. 575, 231-246.
12. Ma, Y., Metch, J.W., Vejerano, E.P., Miller, I.J., Leon, E.C., Marr, L.C., Vikesland, P.J., Pruden, A. Microbial community response of nitrifying sequencing batch reactors to silver, zero-valent iron, titanium dioxide, and cerium dioxide nanomaterials. *Water Research*. 2015. 68, 87-97.
13. Cui, B., Ren, L., Xu, Q.H. Silver nanoparticles inhibited erythrocytogenesis during zebrafish embryogenesis. *Aquatic Toxicology*. 2016. 177, 295-305.
14. Begum, A.N., Aguilar, J.S., Elias, L., Hong, Y. Silver nanoparticles exhibit coating and dose-dependent neurotoxicity in glutamatergic neurons derived from human embryonic stem cells. *Neurotoxicology*. 2016. 57, 45-53.

15. Osborne, O.J., Mukaigasa, K., Nakajima, H. Sensory systems and ionocytes are targets for silver nanoparticle effects in fish. *Nanotoxicology*. 2016. 10, 1276-1286.
16. Minghetti, M., Schirmer, K. Effect of media composition on bioavailability and toxicity of silver and silver nanoparticles in fish intestinal cells (RTgutGC). *Nanotoxicology*. 2016. 10, 1526-1534.
17. Molleman B, Hiemstra T (2017) Time, pH, and size dependency of silver nanoparticle dissolution: the road to equilibrium. *Environ Sci Nano* 4:1314–1327
18. Hume SL, Chiaramonti AN, Rice KP, Schwindt RK, Maccuspie RI, Jeerage KM (2015) Timescale of silver nanoparticle transformation in neural cell cultures impacts measured cell response. *J Nanopart Res* 17:315
19. Tejamaya M, Romer I, Merrifield RC, Lead JR (2012) Stability of citrate, PVP, and PEG coated silver nanoparticles in ecotoxicology media. *Environ Sci Technol* 46:7011–7017
20. Jorge de Souza, T., Rosa Souza, L., & Franchi, L. (2019). Silver nanoparticles: An integrated view of green synthesis methods, transformation in the environment, and toxicity. *Ecotoxicology and Environmental Safety*, 171, 691-700.
21. Cunningham, S., Joshi, L. Assessment of exposure of marine and freshwater model organisms to metallic nanoparticles [WWW Document]. EPA Res. 2015. Rep. 150.
22. SCENIHR Scientific Committee on Emerging and Newly Identified Health Risks SCENIHR Opinion on Nanosilver: Safety, Health, and Environmental Effects and Role in Antimicrobial Resistance. 2014.
23. Liu, J.Y., Hurt, R.H. Ion release kinetics and particle persistence in aqueous nano-silver colloids. *Environmental Science & Technology*. 2010. 44, 2169-2175.
24. Luoma, S.N. *Silver Nanotechnologies and the Environment: Old Problems or New Challenges*. Project on Emerging Nanotechnologies Publication 15 Woodrow Wilson International Center for Scholars and PEW Charitable Trusts, Washington D.C. 2008.
25. Kataoka, C., Kato, Y., Ariyoshi, T., et al., 2018. Comparative toxicities of silver nitrate, silver nanocolloids, and silver chloro-complexes to Japanese medaka embryos, and later effects on population growth rate. *Environ. Pollut.* 233, 1155–1163.
26. Vazquez-Munoz R, Borrego B, Juarez-Moreno K, Garcia-Garcia M, Mota Morales JD, Bogdanchikova N, Huerta-Saquero A (2017) Toxicity of silver nanoparticles in biological systems: does the complexity of biological systems matter? *Toxicol Lett* 276:11–20
27. Asmonaite, G., Boyer, S., de Souza, K.B., et al., 2016. Behavioural toxicity assessment of silver ions and nanoparticles on zebrafish using a locomotion profiling approach. *Aquat. Toxicol.* 173, 143–153.
28. Lee KJ, Nallathamby PD, Browning LM, Osgood CJ, Xu XHN. In vivo imaging of transport and biocompatibility of single silver nanoparticles in early development of zebrafish embryos. *ACS Nano* 2007;1:133–43.
29. Li, Y., Guo, M., Lin, Z., et al., 2016. Polyethylenimine-functionalized silver nanoparticle-based co-delivery of paclitaxel to induce HepG2 cell apoptosis. *Int. J. Nanomed.* 11, 6693–6702
30. Shrivastava, R., Kushwaha, P., Bhutia, Y.C., Flora, S., 2016. Oxidative stress following exposure to silver and gold nanoparticles in mice. *Toxicol. Ind. Health* 32, 1391–1404.
31. Franchi, L.P., Manshian, B.B., de Souza, T.A.J., et al., 2015. Cytotoxic and genotoxic effects of metallic nanoparticles in untransformed human fibroblast. *Toxicol. Vitro* 29, 1319–1331.
32. Asharani, P. V.; Wu, Y. L.; Gong, Z.; Valiyaveetil, S. Toxicity of Silver Nanoparticles in Zebrafish Models. *Nanotechnology* 2008, 19, 1–8.
33. Antsiferova AA, Buzulukov YP, Kashkarov PK, Kovalchuk MV (2016) Experimental and theoretical study of the transport of silver nanoparticles at their prolonged administration into mammal organism. *Crystallogr Rep* 61:1020–1026
34. Stampoulis, D., Sinha, S. K., & White, J. C. (2009). Assay-Dependent Phytotoxicity of Nanoparticles to Plants. *Environmental Science & Technology*, 43(24), 9473–9479
35. Sawosz E, Binek M, Grodzik M, Zielinska M, Sysa P, Szmidi M, et al. Influence of hydrocolloidal silver nanoparticles on gastrointestinal microflora and morphology of enterocytes of quails. *Arch Anim Nutr* 2007; 61:444–51.
36. Yalsuyi, A. M., Vajargah, M. F. Acute Toxicity of Silver Nanoparticles in Roach (*Rutilus rutilus*) and Goldfish (*Carassius auratus*). *Journal of Environmental Treatment Techniques*. 2017. 5 (1), 1-4.
37. Kouhbanani, M. A. J., Beheshtkhou, N., Nasirmoghadas, P., Yazdanpanah, S., Zomorodian, K., Taghizadeh, S., Amani, A. M. Green Synthesis of Spherical Silver Nanoparticles Using *Durossia Anethifolia* Aqueous Extract and Its Antibacterial Activity. *Journal of Environmental Treatment Techniques*. 2019. 7(3), 461-466.
38. Khan, N., Bano, A. Role of plant growth-promoting rhizobacteria and Ag-nano particle in the bioremediation of heavy metals and maize growth under municipal wastewater irrigation. *International Journal of Phytoremediation*, 2015.
39. Evangelou, M.W., Papazoglou, E.G., Robinson, B.H., Schulin, R. Phytomanagement: phytoremediation and the production of biomass for economic revenue on contaminated land Phytoremediation. Springer International Publishing. 2015. 115-132.
40. Padmapriya, S., Murugan, N., Ragavendran, C., Thangabalu, R., Natarajan, D. Phytoremediation potential of some agricultural plants on heavy metal contaminated mine waste soils, Salem District, Tamilnadu. *International Journal of Phytoremediation*, 2015.
41. Eissa, M.A., 2017. Phytoextraction mechanism of Cd by *Atriplex lentiformis* using some mobilizing agents. *Ecol. Eng.* 108 (Part A), 220–226.
42. Adamu, Y.U., Tasiu, S.I., Salisu, M.T. The use of *pistia stratiotes* to remove some heavy metals from Romi stream: a case study of Kaduna refinery and petrochemical company polluted stream. *Journal of Environmental Science, Toxicology and Food Technology*. 2015. 9 (1), 48-51.
43. Gomati, S., Adhikari, S., Mohanty, P. Phytoremediation of copper and cadmium from water using water hyacinth, *Eichhornia crassipes*. *Journal of Agricultural Science and Technology*. 2014. 2(1), 1-7.
44. Harris, A.T., Bali, R. On the formation and extent of uptake of silver nanoparticles by live plants. *Journal of Nanoparticle Research*. 2008. 10(4), 691–695.
45. Andreotti, F., Mucha, A.P., Caetano, C., Rodrigues, P., Gomes, C.R., Almeida, C.M.R. Interactions between salt marsh plants and Cu nanoparticles—effects on metal uptake and phytoremediation processes. *Ecotoxicology and Environmental Safety*. 2015. 120, 303-309.
46. Farkas, J., Peter, H., Ciesielski, T.M., Thomas, K.V., Sommaruga, R., Salvenmoser, W., et al. Impact of TiO₂ nanoparticles on freshwater bacteria from three Swedish lakes. *Science of the Total Environment*. 2015. 535, 85-93.
47. Schaumann, G.E., Philippe, A., Bundschuh, M., Metreveli, G., Klitzke, S., Rakcheev, D., et al. Understanding the fate and biological effects of Ag- and TiO₂-nanoparticles in the environment: the quest for advanced analytics and interdisciplinary concepts. *Science of the Total Environment*. 2015. 535, 3-19.
48. Xu, C., Peng, C., Sun, L., Zhang, S., Huang, H., Chen, Y., Shi, J. Distinctive effects of TiO₂ and CuO nanoparticles on soil microbes and their community structures in flooded paddy soil. *Soil Biology & Biochemistry*. 2015. 86, 24-33.
49. Fernandes, J. P., Mucha, A. P., Francisco, T., Gomes, C. R., Almeida, C. M. R. Silver nanoparticles uptake by salt marsh plants: Implications for phytoremediation processes and effects in microbial community dynamics. *Marine Pollution Bulletin* 2017. 119(1), 176-183.

50. Olkhovych, O., Svetlova, N., Konotop, Y., Karaushu, O.V., & Hrechishkina, S. (2016). Removal of Metal Nanoparticles Colloidal Solutions by Water Plants. *Nanoscale Research Letters*.
51. Bernas, L., Winkelmann, K.D., & Palmer, A.C. (2017). Phytoremediation of Silver Species by Waterweed (*Egeria Densa*).
52. Hanks, N. A., Caruso, J. A., Zhang, P. Assessing *Pistia stratiotes* for phytoremediation of silver nanoparticles and Ag(I) contaminated waters. *Journal of Environmental Management*. 2015. 164, 41-45.
53. Fernandes, J.P., Almeida, C.M.R., Basto, M.C.P., Mucha, A.P., 2015. Response of a saltmarsh microbial community to antibiotic contamination. *Sci. Total Environ*. 532, 301–308.
54. Almeida, C.M.R., Mucha, A.P., Delgado, M.F., Cacador, M.I., Bordalo, A.A., Vasconcelos, M.T.S., 2008. Can PAHs influence Cu accumulation by saltmarsh plants? *Mar. Environ. Res.* 66 (3), 311–318.
55. Kadukova, J., 2010. *Phytoremediation and Stress: Evaluation of Heavy Metalinduced Stress in Plants*. Nova Science Publishers.
56. Sarah, S., Simon, G., 2013. *Calcium. Plant Roots*. CRC Press, 20-21-20-13.
57. Steudle, E., 2000. Water uptake by roots: effects of water deficit. *J. Exp. Bot.* 51 (350), 1531-1542.
58. Koontz, H.V., Berle, K.L., 1980. Silver uptake, distribution, and effect on calcium, phosphorus, and sulfur uptake. *Plant Physiol.* 65 (2), 336 -339.