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Assessment of the Decontamination and Disinfecting Potentials of *Ocimum gratissimum* L. Synthesized Silver Nanoparticles on Water and Wastewater Samples

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Abstract

The presence of organic, inorganic and biological pollutants in natural water supply are the major indices of water pollution. The application of green nanoparticles in water treatment is one of the promising ways of eradicating these pollutants and provide safe and quality water for domestic, agriculture and industrial purposes. Our research was intended to evaluate the decontamination and disinfecting potentials of *O. gratissimum* silver nanoparticle on water and wastewater samples. The green silver nanoparticles were formed using aqueous *O. gratissimum* leaf extract and characterized using standard nanotechnological techniques while decontamination and disinfecting potentials of the green silver nanoparticles was examined through physicochemical, heavy metal and bacteriological analysis. The results revealed the spectral and morphological profiles were in conformity to the characteristics of silver nanocrystals. There were significant reductions ($P < 0.05$) in physicochemical, heavy metal and bacterial qualities of the four water samples at higher (0.5 mg/L) and lower (0.25 mg/L) doses of the green silver nanoparticles and their decontamination and disinfection efficiencies were comparable to the positive control calcium hypochlorite. These findings suggest that *O. gratissimum* silver nanoparticle could be exploited in restoring the quality of water and wastewater.

Keywords: *O. gratissimum, Decontamination, Disinfection, Silver nanoparticles, Water quality*

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Introduction

In the past decades, there has been an alarming increase in the degree of mortality, hospitalization and morbidity which is predicated on the facts that microbial and chemical pollutants are the major and significant attributes of wide range of infectious diseases and health conditions. Reports have it that the human and public health are under serious threats as these pollutants known as pathogens (bacteria, fungi, viruses, algae, and protozoa), heavy metals, organic and inorganic substances could be transmitted through water purification systems, contaminated medical devices, food manufacturing machines and other industrial and domestic activities and materials[1]. Oves *et al*.[2] in their publication reported that in developing countries, approximately 12 million people die annually from drinking of water contaminated with various microbes and chemical contaminants.

Conventionally, water and wastewater are widely treated and disinfected using various physical, chemical and biological processes, the results of which have drawbacks such as high operational cost, labour intensive, lower efficiencies of treatment, stricter legislation and environmental unfriendliness[3]. As of result, there is growing search and interest for alternative and promising decontamination and disinfection techniques.

Presently, the unique features and extensive application of metal nanoparticles especially gold and silver origins, in environmental and medical fields have drawn the attention and awareness of researchers. The biological synthesis of metal nanoparticles is a better alternative to physical and chemical methods owing to the facts that they environmental - friendly, less expensive and less time required[4]. Various substances are classified under the biological nanoparticle synthesis and these include plant extracts, microbes, enzymes and their metabolic by – products[5]. But plant extracts have been the most important among them due to their natural abundance and rich phytochemical ingredients which include terpenoids, polyphenols, sugars, flavonoids alkaloids, phenolic acids, and proteins[6]. In recent times, nanoparticles (NPs) have demonstrated excellent biocidal activities against myriads of microorganisms at different laboratory incubation conditions as well as different water and wastewater with different chemical characters[7].

Ocimum gratissimum L., popularly known as scent leaf, is one of the discovered medicinal plants with the potential to serve as an alternative therapy for the treatment of various ailments or as a source of a new phytocoagulant and disinfectant[8]. Shittu and Ihebunna[4] reported that the *Piliostigma thonningii* synthesized silver nanoparticle also showed heavy metal removal activity in laboratory simulated waste water. Most inhabitants of these studied areas located in Delta States, Nigeria do not have access to potable water and therefore resort to depend on these water sources for domestic, agricultural and industrial activities. Although, literatures exist on the water qualities of these studied areas, there is paucity of any promising treatment regimens.

Also, as far as our knowledge extends, there is no information available on the potential use of *O. gratissimum* L. silver nanoparticles in decontaminating and disinfecting water and wastewater, or any treatment and disinfection techniques associated with it. The present study was therefore undertaken to evaluate the decontamination and disinfecting potentials of *O. gratissimum* silver nanoparticle on water and wastewater samples from Delta States, Nigeria.

Materials and Methods

Plant collection and extract preparation

The plant leaves of *O. gratissimum* were obtained from the local market around the Ogwashi - Kwu Campus of Delta State Polytechnic, Nigeria. The plant specimen was recognized by a botanist Prof. Ukpaka, Chukwujekwu from Department of Biological Sciences, Chukwuemeka Odumegwu Ojukwu University. The fresh leaves of *O. gratissimum* were washed with clean water, and then air - dried for 2 weeks at room temperature. The leaves were destalked and crushed into powdery form using industrial blender. Twenty-five (25 g) of the powdered leaves of *O. gratissimum* was weighed, mixed and a boiled for 25 min with 500 mL distilled water. The aqueous extract was double filtered and filtrate was used for the biosynthesis of silver nanoparticles[4].

Qualitative phytochemical screening

The descriptive method of Okaiyeto *et al.*[9] was adopted for the qualitative determination of phytochemicals ingredients of the plant aqueous extract.

Biosynthesis of silver nanoparticles (NPs)

The method of Shittu and Ihebunna[4] was adopted silver nanoparticle synthesis by mixing 5 mL *O. gratissimum* of aqueous extract with 95 mL of aqueous solution of 1 mM $AgNO_3$ and heated with stirrer at 70 °C for 60 min and pH 7 till colour change is observed.

Characterization of the green silver nanoparticles

The physical and chemical characteristics of the synthesized green silver nanoparticles were determined using standard nanotechnological techniques as described by Shittu and Ihebunna[4]

Sampling area description and collection of water sample

The studied areas include well water, water shed and stream water in Ogwashi Ukwu in Aniocha South LGA, Delta State and river water of River Niger in Onitsha South LGA, Anambra State Nigeria, respectively. The water samples were collected using 2 L dark ambered bottles, mixed together as composite samples of each site, appropriately labelled, placed in ice- parked coolers and taken to the laboratory for the physicochemical and bacterial analyses[10].

Physicochemical analysis

The following parameters such as temperature, pH, turbidity, conductivity, chemical oxygen demand (COD), sulphate, total phosphate and total chloride was analyzed using standard procedures of APHA[11] and AOAC[12].

Total heavy metal analysis

The untreated and treated water samples were analyzed for their total heavy metal contents using atomic absorption spectrophotometer such as cobalt (Co), copper (Cu), lead (Pb), cadmium (Cd), chromium (Cr), zinc (Zn), nickel (Ni), mercury (Hg) and arsenic (As) according to the standard method of APHA[11]. The percent metal removal or reduction was obtained using the equation below:

$$\%\ \text{Metal reduction} = \frac{\text{Initial metal concentration} - \text{final metal concentration}}{\text{Initial metal concentration}} \times \frac{100}{1}$$

Total bacterial analysis

The standard spread plate technique was adopted for the quantification of the total bacterial contents of the untreated and treated water samples using Nutrient Agar (NA). After 24 h incubation for total bacteria at 37 ± 2 °C, the emerging colonies were counted and expressed as colony forming unit per milliliter (CFU/mL)[7].

Decontamination and disinfection study

The effects of the produced *O. gratissimum* AgNPs and chlorine solution on the physicochemical, heavy metal and bacterial contents of various water and wastewater samples were determined in the decontamination and disinfection study. In this study, the samples were treated with 0.25 and 0.5 mg/mL of the sterilized produced NPs (passed through 0.22 µm pore size) for 2 h as a contact time while the standard chlorine doses of 5 mg/L and untreated samples serve as positive and negative controls. The whole experiment will be conducted in pre-sterilized 1 L glass amber bottles at room temperatures 25 ± 2 °C and repeated in triplicates for each sample. At the end of the 2 h contact time, the physicochemical, heavy metal and microbial contents were determined from the respective sample setups as previously described above and compared with the controls using the following expressions below and according to the methods of Eltarahony *et al.*[7] and Shittu and Ihebunna[4]:

$$\% \text{ Inhibition of bacteria/fungi} = \frac{\text{CFU in control} - \text{CFU of colonies in treatment}}{\text{CFU in control}} \times \frac{100}{1}$$

CFU = Colony forming units; % = Percent.

Biostatistical management

The data obtained were statistically described in mean and standard deviation. The mean data were simultaneously subjected to two-factor analysis of variance (ANOVA) and Tukey multiple comparison test using GraphPad Prism Version 8.0.2. Statistical significance was set at the probability level less than 0.05[4].

Results and Discussion

The synthesis of green nanoparticles has been a growing research area in the scientific world over the last decades utilizing the potentials of different plant species, parts and extracts in the nanoparticle biosynthesis. In this study, the phytochemical ingredient of *O. gratissimum* leaf extract is presented in Table 1 and it revealed the presence of flavonoid, coumarin, anthraquinone, phenol, saponin, and phytosteroid, alkaloid and tannin while it also revealed the absence of quinone, terpenoid, cardiac glycoside and steroid. Different biological activities such as antimicrobial, antioxidant, anticancer et cetra have been attributed to this presence of these phytochemicals detected in this study[9]. Several researches carried out by Bawazeer *et al.*[13], Alharbi and Alsubhi[14], Alharbi *et al.*[15], Chakravarty *et al.*[16] and Rani *et al.*[17] have implicated the potentials of leaves of *Azadirachta indica*, *Tropaeolum majus*, *Cassytha filiformis* L. and *Syzygium cumini* in silver nanoparticle synthesis. Also, earlier reports revealed the abilities of these phytochemicals in the bioreduction of metals as well as stabilization of the formed particles[9].

Table 1: Phytochemical content of *O. gratissimum* leaf extract

Parameter	Observation
Flavonoid	++
Quinone	-
Coumarins	++
Alkaloid	+
Anthraquinone	++
Terpenoid	-
Phenol	++
Saponin	++
Phytosteroid	++
Cardiac glycoside	-
Steroid	-
Tannin	+

++ = Presence, + = Trace, - =Absence

The visual colour changes and UV-Vis spectroscopy were used to monitor the development in the AgNPs synthesis[18]. In this study, the colourless solution of the precursor ($AgNO_3$) was converted to dark brown colouration indicating the reaction completion and excitation of the surface plasmon resonance (SPR) and agreed with the published works of Okaiyeto *et al.*[9] and Vanlalveni *et al.*[18]. Subsequently, the confirmation of the formation of the Ag/AgCl NPs was carried out with UV-Vis spectroscopy and the result UV – Vis spectra profile as demonstrated in Figure 1 revealed that the highest spectral absorption peak was 400 nm. This sharp peak value was attributed to the SPR which is an excitational phenomena of the electromagnetic interaction between the electrons of the Ag/AgCl NPs and certain wavelengths. Our result is in conformity with several scientific reports using *O. gratissimum* aqueous extract whose SPR peaks were found to be 400 – 600 nm, respectively[13,19, 21] but contradicts the findings of Okaiyeto *et al.*[9] and Larayetan *et al.*[22] and the contradiction could be due to differences in plant species. Also, there the observation of more than one peaks in the absorption spectra revealed that the biogenically formed Ag/AgCl NPs are anisotropic particles and therefore polydisperse and so agreed with the report of Vanlalveni *et al.*[18]

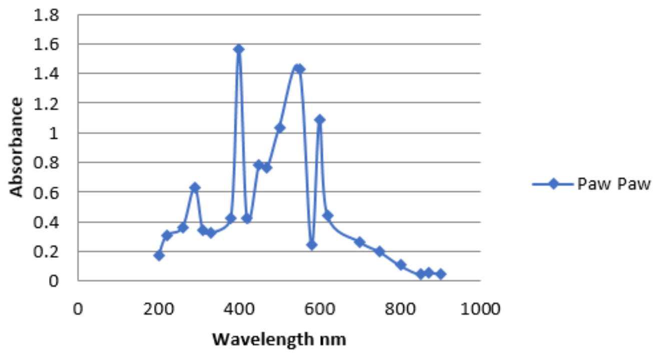

Figure 1: UV – Vis spectra profile of *O. gratissimum* synthesized silver nanoparticles

X – ray diffraction (XRD) analysis is widely used to demonstrate the specific and crystalline nature of the particles. In this study, different patterns of XRD were produced when a monochromatic beam of X-rays were absorbed by crystal samples, and results analyzed using Bragg's equation in order to reveal the exceptional crystal features of the produced Ag/AgCl NPs[9]. Table 2 showed the result of the XRD feature of the green silver nanoparticle using *O. gratissimum*. The results revealed that there are six intense peaks in the whole spectrum of two-degree theta shift data ranging from 30° to 70°, respectively. The obtained XRD patterns in this study were compared with the known reference data published by the Joint Committee on Powdery Diffraction Standards (JCPDS) (File. No. 04-0783). It was found that our green silver nanoparticles are in conformity to the nanocrystals database, as evidenced by the peaks at $2\theta°$ = 38.0544°, 39.8185°, 44.2916°, 57.3933°, 64.5287° and 68.8359°, corresponding to crystallographic (1 1 1) (1 1 1) (2 0 0) (2 1 1) (2 2 0) and (2 2 1) lattice planes of face-centered cubic (FCC) structure of metallic silver. Similar results were obtained by the study carried out by Sharma *et al.*[23], Chandrasekar and Kannaiyan [24] and Fozia *et al.*[25]. The mean diameter of the silver nanoparticles was calculated from the XRD patterns and the average size of the particle was determined using Debye-Scherrer Equation. It was discovered that the *O. gratissimum* synthesized nanoparticles had an average particle size of 35.03 nm. The definitive nanoscale has been reported to range from 1 nm to 100 nm for nano - synthesized materials [20] and upholds the result obtained this study.

The scanning electron microscope (SEM) analysis is widely in nanotechnological study for characterization of surface morphology and topography. In this study, the result of the scanning electron micrograph of *O. gratissimum* synthesized silver nanoparticle is shown in Figure 2. From the Figure 2, it showed a high density of spherical loosely bound silver nanoparticles and polydispersed particles without agglomeration. Surara *et al.*[20] reported that their SEM result of *O. gratissimum* synthesized observed a high density and aggregation of spherical AgNPs at the 200 nm scale. The shape obtained in this study is similar to the observations of Kumavat and Mishra[26], Sharma *et al.*[23], Sani *et al.*[27] and Basalius *et al.*[28].

Table 2: XRD feature of the green silver nanoparticle using *O. gratissimum*

Pos. [°2Th.]	Height [cts]	FWHM Left [°2Th.]	d-spacing [Å]	Relative intensity [%]	Average size	Miller indice
38.0544	560.06	0.1378	2.36471	100.00	61.00	111
39.8185	14.72	0.2362	2.26393	2.63	35.60	111
44.2916	89.96	0.1968	2.04512	16.06	43.60	200
57.3933	3.42	0.9446	1.60554	0.61	9.58	211
64.5287	29.06	0.3149	1.44417	5.19	29.80	220
68.8359	11.73	0.3149	1.36395	2.09	30.6	221
					Average size 35.03	

Key: % = Percentage, cts = Counts, Th = Theta, Å = Angstrom, nm = Nanometre

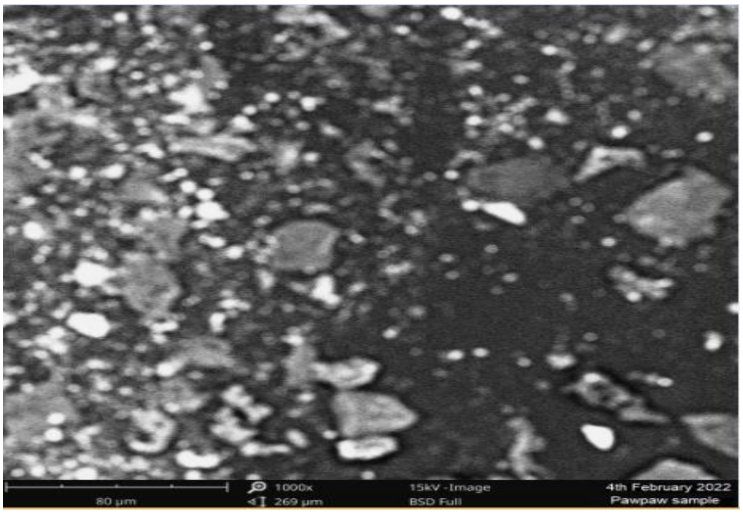

Figure 2: Scanning electron micrograph (1000x) of *O. gratissimum* synthesized silver nanoparticle

The challenge of water scarcity especially in developing countries like Nigeria could be tackled through cheap and ecofriendly decontamination and disinfection processes of water and wastewater in order to achieve the Millennial goal of water quality standards[29]. In the present study, Table 3 presents the effect of calcium hypochlorite and green silver nano particle on physicochemical properties of the water and wastewater sample and it demonstrated that pH is acidic to alkalinity (4.80±0.000 - 8.10±0.000). Liu *et al*.[30] reported pH range of 6.5 - 8.0 for the decontamination of chemical contents of wastewater. Also, moderate in temperature range (29.00±0.002 - 32.00±0.006 °C) was observed during the treatment period. Conductivity was found to be in increasing range (0.01±0.000 - 4.80±0.001 mS/cm^2) in all the exposed water samples. Many authors reported that nanoparticles of silver metal help to progress the electrical conductivity of different liquid media[6,31]. The results revealed significant decreasing trend range in turbidity (89.46±0.010 - 0.00±0.001 NTU), sulphate (64.31±0.010 - 0.26±0.010 mg/L), phosphate (985.65±0.020 - 29.57±0.001 mg/L), COD (672.00±0.012 - 0.26±0.001 mg/L) and chloride (4165.38±0.040 - 233.35±0.020 mg/L) contents of all the exposed water samples under 5.0 mg/L $CaOCl_2$, 0.25 mg/L and 0.5 mg/L treatment regimens during the 2 h period. The reason for this decrease in nitrate and phosphate contents of the treated water samples could be due to nitrification and phosphorylation inhibition activities of the *O. gratissimum* silver nanoparticles and agreed with scientific reports of Kamal *et al*.[6] and Zhang *et al*.[32]. Significant differences were detected only among the means of turbidity, sulphate, phosphate, COD and chloride contents and their controls, respectively. The decontamination efficiencies of *O. gratissimum* silver nanoparticles were almost comparable to the commercially available decontaminating agent calcium hypochlorite. Shittu *et al*.[33] reported that wastewater treated with the synthesized SNPs and subjected to physicochemical analysis revealed the ability of the SNPs to remove pollutants and upheld the findings in this study.

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Table 3: Effect of calcium hypochlorite and green silver nanoparticle on physicochemical properties of the water and wastewater sample

Water source	Decontam-inating agent	Physicochemical properties							
		pH	**Temp (ºC)**	**Con (mS\cm²)**	**Turb (NTU)**	**SO_4 (mg\L)**	**PO_4 (mg\L)**	**COD (mg\L)**	**Cl (mg\L)**
Water shed	Untreated	6.90±0.001	32.00 ± 0.003	0.18±0.000	89.46±0.010	26.69±0.020	985.65±0.020	672.00±0.12	3,669.00±0.20
	5.0 mg/L $CaOCl_2$	7.10±0.001	31.00±0.002	1.06±0.005	60.23±0.050	02.88±0.040	801.30±0.030	262.40±0.010	6115.13±0.010
	0.50 mg/L OGSNP	4.90±0.000	32.00±0.001	0.19±0.003	23.31±0.070	20.78±0.060	349.56±0.060	0.73±0.001	2658.75±0.020
	0.25 mg/L OGSNP	4.80±0.000	32.00±0.000	0.20±0.001	77.92±0.010	21.45±0.080	350.73±0.017	0.60±0.040	3208.23±0.060
Stream water	Untreated	8.00±0.003	30.00±0.001	0.01±0.000	35.23±0.000	32.17±0.020	296.65±0.000	438.40±0.020	3988.13±0.30
	5.0 mg/L $CaOCl_2$	7.90±0.001	30.00±0.002	4.80±0.001	0.00±0.001	4.07±0.030	228.90±0.040	352.80±0.040	6132.85±0.090
	0.50 mg/L OGSNP	7.30±0.002	31.00±0.003	0.01±0.003	11.46±0.040	0.52±0.010	29.57±0.001	0.38±0.060	233.35±0.020
	0.25 mg/L OGSNP	7.20±0.003	30.00±0.004	0.01±0.005	0.00±0.020	0.26±0.010	70.81±0.070	26.50±0.080	276.50±0.020
River water	Untreated	7.90±0.004	30.00±0.005	0.09±0.007	50.50±0.060	64.31±0.010	575.65±0.006	544.00±0.0400	3633.63±0.010
	5.0 mg/L $CaOCl_2$	8.70±0.001	30.00±0.007	0.09±0.004	37.5±0.001	1.69±0.040	79.13±0.002	342.40±0.050	5406.30±0.030
	0.50 mg/L OGSNP	7.80±0.001	32.00±0.006	0.10±0.003	0.68±0.000	39.30±0.001	204.65±0.006	0.72±0.001	3266.43±0.010
	0.25 mg/L OGSNP	6.70±0.001	31.00±.0001	0.05±0.002	21.35±0.030	7.60±0.001	187.39±0.001	0.45±0.010	1896.56±0.050
Well water	Untreated	8.10±0.001	30.00±0.004	0.11±0.001	22.46±0.001	8.12±0.040	306.43±0.003	560.00±0.040	4165.38±0.040
	5.0 mg/L $CaOCl_2$	8.10±0.000	30.00±0.001	0.18±0.000	15.54±0.020	0.81±0.010	47.39±0.006	310.40±0.030	7061.38±0.030
	0.50 mg/L OGSNP	8.10±0.006	29.00±0.002	0.12±0.000	0.54±0.000	2.52±0.020	60.00±0.001	0.66±0.010	3545.00±0.060
	0.25 mg/L OGSNP	8.10±0.005	30.00±0.003	0.11±0.000	0.23±0.000	0.50±0.010	70.44±0.001	0.26±0.010	2589.15±0.080

Key: $CaOCl_2$ = Calcium hypochlorite; mg/L = Milligram per liter; OGSNP = *O. gratissimum* silver nanoparticle; Con = Conductivity; mS\cm² = MilliSiemens per square centimetre; Temp = Temperature; ºC = Degree centigrade; Turb = Turbidity; NTU = Nephelometric Turbidity Unit; SO_4 = Sulphate; PO_4 = Phosphate; COD = Chemical oxygen demand Cl = Chloride

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The major indicators of water contamination are the occurrence of microorganisms and heavy metals. Several health hazards that are of public health priority list are linked to heavy metal pollution of water. In this study, Table 4 (see supplementary file) present the removal percentage of heavy metal of the different water sample at different concentration of calcium hypochlorite and green silver nano particle. The results demonstrated significant removal percentage range of 10.60±0.020 - 99.50±0.020 % for Hg; 10.60±0.130 - 100.00±0.050 % for Cu; 09.23±0.070 - 97.85±0.010 % for Zn; 33.33±0.040 - 98.18±0.040 % for Pb; 05.88±0.090 - 98.00±0.040 % for Cd; 14.29±0.080 - 96.67±0.010 % for Co; 25.94±0.010 - 100.00±0.100 % for As; 20.30±0.070 - 99.75 %±0.030 for Ni and 20.00±0.020 - 95.75±0.004 % for Cr, respectively. The reason for these efficient removing or adsorption capabilities of these heavy metals could be due to the unique physical and chemical properties (very high surface area, high surface-area-to volume ratios, high reactivities, smaller sizes, specific affinities) of the biogenic silver nanoparticles[3]. The 0.25 mg/L nanoparticle treatment regimen had more removal percentages than the 0.5 mg/L nanoparticle treatment regimen and was comparable to the to the commercially available decontaminating agent calcium hypochlorite. Statistically, non - significant differences ($P > 0.05$) were obtained between 0.5 mg/L concentration and the positive control calcium hypochlorite while significant differences ($P < 0.05$) were obtained between the 0.25 mg/L concentration and the positive control calcium hypochlorite among the percentage means of heavy metals analyzed. Previous study by Shittu and Ihebunna[4] reported that biosynthesized silver nanoparticles using *Piliostigma thonningii* have efficient ability in heavy metal removal with 82.1 % for copper, 96.9 % for iron and 97.89 % for lead after 60 min optimized time, respectively and agreed with the observations made in this study.

Table 4: Percentage removal of heavy metal of the different water sample at different concentration of calcium hypochlorite and green silver nanoparticle

Water source	Decontaminating agent	Metal removal percentage (%)								
		Hg	Cu	Zn	Pb	Cd	Co	As	Ni	Cr
Stream water	0.50 mg/L OGSNP	10.60±0.020	10.60±0.130	09.23±0.070	94.22±0.020	78.67±0.010	94.47±0.200	54.84±0.080	26.47±0.090	95.75±0.004
	0.25 mg/L OGSNP	57.83±0.120	64.82±0.030	61.54±0.050	33.33±0.040	33.33±0.030	64.67±0.020	55.81±0.060	63.82±0.070	67.50±0.010
	5.0 mg/L $CaOCl_2$	55.30±0.010	41.71±0.020	47.23±0.030	73.33±0.060	80.00±0.050	78.00±0.040	85.48±0.040	57.58±0.050	85.83±0.020
Water shed	0.50 mg/L OGSNP	69.74±0.001	64.46±0.010	43.75±0.020	41.18±0.080	53.95±0.070	53.95±0.060	75.76±0.020	07.41±0.030	07.41±0.030
	0.25 mg/L OGSNP	36.84±0.040	81.11±0.001	11.11±0.020	84.50±0.010	05.88±0.090	14.29±0.080	100.00±0.100	23.33±0.010	87.41±0.050
	5.0 mg/L $CaOCl_2$	80.26±0.060	76.06±0.001	23.53±0.010	60.44±0.030	58.82±0.010	66.67±0.010	85.48±0.090	75.00±0.010	22.22±0.070
Well water	0.50 mg/L OGSNP	20.50±0.080	18.18±0.002	69.29±0.080	55.00±0.050	55.00±0.020	80.00±0.020	88.46±0.070	85.00±0.020	76.42±0.090
	0.25 mg/L OGSNP	99.50±0.020	77.93±0.006	76.53±0.060	35.00±0.070	98.00±0.040	76.00±0.090	88.46±0.050	99.75±0.030	47.55±0.100
	5.0 mg/L $CaOCl_2$	63.00±0.030	99.73±0.080	60.00±0.040	85.00±0.090	86.60±0.060	47.00±0.070	30.23±0.030	75.00±0.040	87.05±0.080
River water	0.50 mg/L OGSNP	28.00±0.010	69.44±0.100	81.01±0.020	68.15±0.020	60.87±0.080	48.00±0.050	28.13±0.010	66.67±0.050	15.67±0.060
	0.25 mg/L OGSNP	91.56±0.001	95.28±0.040	97.85±0.010	98.18±0.040	96.52±0.100	86.00±0.030	25.94±0.020	95.45±0.060	56.67±0.040
	5.0 mg/L $CaOCl_2$	74.70±0.070	100.00±0.050	58.23±0.010	84.55±0.060	81.82±0.030	96.67±0.010	56.63±0.010	20.30±0.070	20.00±0.020

Keys: Hg = Mercury, Cu = Copper, Zn = Zinc, Pb = Lead, Cd = Cadmium, Co = Cobalt, As = Arsenic, Ni = Nickel, Cr = Chromium, % = Percentage, $CaOCl_2$ = Calcium hypochlorite; mg/L = Milligram per liter; OGSNP = *O. gratissimum* silver nanoparticle.

The disinfecting potential of the silver nanoparticle as a promising technology in treatment of natural polluted waters and wastewaters has been the subject of research in recent years and in this study, the result of the percentage inhibition of total bacteria before and after treatment with calcium hypochlorite and green silver nano particle is presented in Table 5 (see supplementary file). The result revealed that higher concentration (0.5 mg/L) had more non -significant ($P > 0.05$) disinfecting effect as evident in bacterial growth percentage inhibition (98.88±1.100 %) than lower concentration (0.25 mg/L) with 96.25±1.500 % bacterial growth percentage inhibition in contrast to the count in untreated control ranging from the 300 – 800 x 10^3 CFU/mL in the water and wastewater samples, respectively. It was also found that both disinfecting doses were almost comparable ($P > 0.05$) to the positive control (calcium hypochlorite) as good disinfectants. The reason for this excellent disinfecting abilities by the particles could be due to the interaction between silver ions releases from the silver nanoparticles and the bacterial cell wall leading to impairment of cell wall and possible cell lysis and death and agreed with the published research studies of Kamal *et al.*[6], Vanlalveni *et al.*[18], Sani *et al.*[27], Jalab *et al.*[34], Wattimena *et al.*[35] and Kumavat and Mishra[36]. Consequently, it has suggested that the nature of the samples and their water qualities should be the basis of designing the effective dose that would eradicate bacterial of public health concern in natural waters[7]

Table 5: Percentage inhibition of total bacteria before and after treatment with calcium hypochlorite and green silver nano particle

Water sample	Untreated (control) (CFU/mL X 10^3)	Disinfecting agent	Treatment dose mg/L (CFU/mL x 10^3)			Percentage growth inhibition (%)		
			5	**0.5**	**0.25**	**5**	**0.50**	**0.25**
Stream water	600	OGSNP	-	40.00±5.000	120.00±14.600	-	93.33±1.600	80.00±2.000
		$CaOCl_2$	10.00±2.200	-	-	98.33±1.300	-	-
Water shed	300	OGSNP	-	50.00±4.500	110.00±10.100	-	83.33±2.200	63.33±1.400
		$CaOCl_2$	18.00±3.100	-	-	94.00±1.200	-	-
River water	800	OGSNP	-	10.00±1.000	30.00± 5.200	-	98.88±1.100	96.25±1.500
		$CaOCl_2$	15.00±1.100	-	-	98.13±2.100	-	-
Well water	350	OGSNP	-	10.00±3.000	30.00± 3.100	-	97.14±1.200	91.43±2.100
		$CaOCl_2$	12.00±2.000	-	-	96.57±1.000	-	-

Key: - = Undetermined, CFU/mL = Colony forming unit per millilitre, % = Percentage, $CaOCl_2$ = Calcium hypochlorite; mg/L = Milligram per liter; OGSNP = *O. gratissimum* silver nanoparticle

Conclusion

The whole study showed that simplicity, cost effective and ecofriendly synthesis of green *O. gratissimum* silver nanoparticle in natural water treatment in addition to their decontamination and disinfecting efficiencies. Both higher (0.5 mg/L) and lower (0.25 mg/L) doses of the *O. gratissimum* silver nanoparticle were found to effectively decontaminate and disinfect the four water samples with regards to physicochemical, heavy metal and bacterial qualities and these abilities were comparable to the positive control calcium hypochlorite.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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Conflicts of Interest

The authors declare no conflict of interest and the funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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