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Biosynthesis and Characterization of Lead Sulfide Nanoparticles Using Wastewater Bacteria

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ABSTRACT

Background: Wastewater considered as a lost economical value, due to it contains a huge content of bacteria that could biosynthesis of a valuable materials from hazardous material. Heavy metals that found in wastewater considered as row material for bacteria to biosynthesis nanoparticles as lead sulfide (PbS) which used as semiconductor**.** ***Aim:*** The present study aims to biosynthesis and characterization of lead sulfide nanoparticles using bacteria isolated from wastewater ***Materials & Methods:*** Sampling different samples from three places at different stages in the wastewater plant. Samples were collected from influent (raw sewage), from the outlet of the 2ry sedimentation tank (after biological treatment) and from the outlet after chlorination (effluent). Determine of physico-chemical characteristics of the wastewater samples. Isolate some of bacteria present in these different stages of treatment in the plant. Study the capabilities of the isolated strains for lead resistance. Determine the minimum inhibitory concentration of selected bacterial strains that are resist to lead concentration. Biosynthesize lead nanoparticles using the selected bacterial isolate. Characterize the lead nanoparticle produced by selected bacterial isolate. ***Results:*** The biosynthesis of lead nanoparticles by *Serratia plymuthica*, which isolated from Zenine wastewater treatment plant, Giza, Egypt. *Serratia plymuthica* had ability to resist lead till 80mg/l and had ability to biosynthesize lead nanoparticle. UV-Vis spectroscopy results for pellets of *Serratia plymuthica* inoculated in Tryptic soy bean broth (TSB) containing 80 mg/l of $Pb(NO_3)_2$show formation of peak at ~ 330 nm, which was a specific peak for lead nanoparticles. TEM image for pellets of *Serratia plymuthica* inoculated in TSB containing 80 mg/l of $Pb(NO_3)_2$show formation of Pb NPs intracellular and extracellular and cells aggregation. Dynamic light scattering (DLS) results show ability of *Serratia plymuthica* to biosynthesis PbNPs with mean diameter 92.93 nm. X- ray diffraction (XRD) results show ability of *Serratia plymuthica* to biosynthesis PbS which had semiconducting properties and used in solar cells manufacturing. **Conclusions:** The results approved the biosynthesis of PbS nanoparticles by *Serratia plymuthica* which isolated from wastewater.

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INTRODUCTION

Waste water was known as any water that has been adversely affected in quality by anthropogenic activities. It comprises liquid waste discharged by domestic residences, commercial properties, small scale industries and institutions. In general, waste water is characterized based on its bulk or organic contents, physical characteristics and specific contaminants [1]. Wastewater environment is an excellent media for a wide range of microorganisms specially bacteria, viruses and protozoa. The majority of microorganisms is harmless and can be used in biological sewage treatment. Also, wastewater contains harmful microorganisms, which are drained to wastewater by sick individuals and a symptomic carrier. Bacteria which cause typhoid, cholera and tuberculosis; viruses which cause infectious hepatitis; protozoa which cause dysentery and the eggs of parasitic worms are all found in sewage [2].

Wastewater contains huge numbers of living organisms ranging from too small to be visible, which is why they are called "microorganisms". Typically, wastewater) ions were reported to be the main heavy-metal ions in the wastewater due to the battery factories [5]. The toxicity of heavy metal was characterized by their cationic superiority and ability to substitute functional ions within the [6]. So, microorganisms develop different detoxification mechanisms as precipitation, sorption efflux system and biotransformation to survive [7, 8].

Many reports in recent years mentioned using of wastewater as raw material for producing a valuable material as bacterial extracellular polymeric substances (EPS) and microbial lipid [9, 10]. Biosynthesis of nanoparticles is gaining importance though recent decade as biological methods comprise clean, nontoxic, and environmental friendly procedures for the synthesis and assembly of nanoparticles. Biological method utilizes nature's most efficient machines i.e. living cells for the synthesis [11].

prior to entering the treatment plant will contain from 100,000 to 1,000,000 microorganisms per milliliter. These microbes have their origin from two general sources: sanitary wastes and the soil. Both wastewaters and soils contain large numbers of microorganisms. Generally, the microorganisms can be considered as a natural living part of the organic matter found in wastewaters and their presence is most important because they serve a primary function in the treatment in biological wastewater treatment. In a sense the successful operation of a biological wastewater treatment plant is dependent upon knowledge of the activities of the microorganisms [3].

Heavy metals are increasingly found in microbial habitats due to several natural and anthropogenic processes; therefore, microbes have evolved mechanisms to tolerate the presence of heavy metals by efflux, complexation, or reduction of metal ions or to use them as terminal electron acceptors in anaerobic respiration [4]. Lead (II), Copper (II) and Cadmium (II

Nanoparticles biosynthesis by microbes has emerged as a promising field of research, with Nano-biotechnology interconnecting biotechnology and nanotechnology [12]. Generally, chemical and physical methods for nanoparticles synthesis are quite expensive and potentially hazardous to the environment which involve use of toxic and perilous chemicals that are responsible for various biological risks. The development of biologically-inspired experimental processes for the syntheses of nanoparticles is evolving into an important branch of nanotechnology Fig. 1 [13].

The biosynthesis of semiconductor nanocrystallites by microorganisms is relatively unexplored and new. Among the first reports of intracellular semiconductor nanoparticle synthesis *Torulopsis sp* has ability to synthesize lead sulfide (PbS)

nanoparticles when challenged with lead [14].

The intracellular synthesis of stable lead sulfide nanoparticles by a marine yeast, *Rhodosporidium diobovatum* [15].

Enterobacter sp and *Bacillus anthracis* were isolated from industrial wastewater and studied the ability of them to produce the lead nanoparticles [16]. [17] synthesized extracellular metal nanoparticles(NPs) silver, lead and cadmium by using *Bacillus megaterium* strain.

The importance of PbS nanoparticles and its application in solar cells, solar absorbers, LED devices, laser, photographs, telecommunication optical switches, optical amplification, detectors and gas- sensing agents in the solid-state sensors were reported by [18].

In this study, we isolated a bacterium from Zenine wastewater treatment plant, Giza, Egypt which have ability to resist the occurrence of heavy metal to use it in the biosynthesis of lead sulfide nanoparticles

Materials and Methods

Material

All chemical materials were analytical grade supplied from Sigma_Aldrich-Germany, Loba- India and Oxford-India.

All microbiological media were supplies from Lab_M-England.

Sites of Wastewater Samples

The samples were taken from three sites representing the main different stages in the wastewater plant. Samples were collected from influent (raw sewage), from the outlet of the 2ry sedimentation tank (after biological treatment and from the outlet after chlorination (effluent) Fig. 2. The day air temperature was ranged from 15 to 25°C.

Sampling and Sample Processing

Samples for chemical examinations were collected manually by manual sampler, which collected a grab sample in a 2 liters non-sterilized glass bottle. The samples were then transferred to the laboratory and examined within 1 hour of collection. The samples were kept constantly homogenized and aerated for the entire duration of analysis, to keep all the solids in suspension. Samples for microbiological examinations were collected manually in sterile 100ml non-reactive borosilicate glass bottles. All samples were stored in a refrigerator at 4ºC till used for microbial experiments.

Physico-chemical Characteristics of Waste Water

pH, total dissolved solid (TDS) and electrical conductivity (EC)

The pH values which demonstrate the intensity of the basic or acid strength of waste samples was measured by electrometric pH meter model Orion 2 STAR, Thermo scientific, USA. Total dissolved solid (mg/l) and electrical conductivity of water sample were measured by conductivity meter model Orion 5 STAR, Thermo scientific, USA [20].

Total suspended Solids (TSS), ammonia nitrogen (NH3) and phosphate

TSS, Ammonia nitrogen (NH3) and Phosphate for wastewater samples were measured according to APHA Standard Methods for the Examination of Water and Wastewater [21]. Procedure number 2450 D, procedure number 4500 D and procedure number 4500-P respectively.

Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD5) and Oil and grease

Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD5) and Oil and grease were measured according to APHA Standard Methods for the Examination of Water and Wastewater [21]. Procedure number 5520 A, procedure number 5210 B and procedure number 5520 B. Partition-Gravimetric method respectively.

Heavy metal

The heavy metals (Cd, Cr, Cu, Fe, Ni and Pb) were measured in water samples after digestion in concentrated HNO_3 which was supplied from Sigma-Aldrich, Germany. Samples were measured according to APHA Standard Methods for the Examination of Water and Wastewater [21].

Procedure number 3120 B by PerkinElmer ICP-OES model Vista Pro.

Microbiological Analysis

Enumeration and isolation of wastewater bacteria

Pour plate method technique according to APHA Standard Methods for the Examination of Water and Wastewater [21]. procedure number 9215 B. Tryptic soy agar media TSA was used to facilitate vigorous growth of aerobic and anaerobic microorganisms, used for the cultivation of a wide variety of microorganisms [22].

Characterization of the bacterial isolates

Different representative colonies were selected from the plates and repeatedly streaked onto the same medium in order to obtain pure cultures. The pure isolates subcultured on slants of the same medium and preserved as glycerol stocks at -20°C for further studies. The bacterial isolates were characterized by shape, edge, color, elevation and gram stain [23].

Selection of most lead ions tolerant bacteria for further studies

The isolates were initially subjected to a study of lead ions resistance. For this, one loopful of microbial growth obtained on TSA plates after 48 h incubation was inoculated by streaking on TSA medium contaminated with 10 ppm for Pb(II) (as $Pb(NO_3)_2$). Plates were incubated at 33°C for 72 hours and visually inspected for microbial growth every day [24]. Strains resistant to the lead metal were subjected to biochemical identification, Minimum Inhibitory Concentration (MIC) and biosynthesis of lead nanoparticle.

Identification of the most lead resistant isolates

The most five potent isolates in lead resistant were identified by biochemical tests via strip system (BioMereux) on Vitek 2C which give gave rapid, reliable, and highly reproducible results [25].

Determination of Minimum Inhibitory Concentration (MIC) for isolate number 29 to lead ions

Minimum Inhibitory Concentration (MIC) of the lead ions resistant bacteria isolate was determined by gradually increasing the concentration of lead ions by 5 mg/L each time on the TSA agar plate until the strains failed to give colonies on the plate. Minimal inhibitory concentration was noted when the isolate failed to grow on the plates after incubation [26].

Synthesis of Lead Nanoparticles

Preparation of seed culture

A loopful of inoculum from the isolate number 29 was inoculated into the Tryptone soy broth (TSB) followed by incubation at 35 ºC and 120 rpm. Twenty-four hour grown culture having OD ˜1.0 at 600 nm using PerkinElmer UV/VIS Spectrometer-Lambda 25 was used as seed culture [11].

Preparation of bacterial culture

1liter of TSB media was inoculated with 1% seed culture and incubated at 35ºC with constant shaking at 120 rpm in orbital rotary shaker for 24h. The bacterial biomass was then harvested from the medium by centrifugation using Thermo megafuge at 7000 rpm (5533 X g) for 10 min. The supernatant was separated. The cell pellet was washed twice with autoclaved with saline (0.85 % NaCl, W/V) and finally with deionized water [16].

Synthesis of lead nanoparticle

To 100 mL of tryptic soy bean broth media containing 80 **ppm** lead nitrate ($Pb(NO_3)_2$), 1 g (wet weight) of washed cells of bacterial isolate was added under **aseptic conditions**. To 100 mL tryptic soy bean broth, 1 g (wet weight) of washed cells of bacterial isolate was added under aseptic conditions as control. The flasks were incubated at 35°C for 72 h. A change in the color of the content of the bottles containing the lead salts gives a preliminary indication of nanoparticles synthesis. The biomass or residue was separated from each bottle by centrifugation at 7000 rpm using Thermo megafuge and collected for further characterization [11].

Characterization of Lead Nanoparticles

UV-Vis Spectroscopy

The synthesized nanoparticle suspended in deionized water was scanned from 200-400 nm using PerkinElmer UV/VIS

Spectrometer- Lambda 25 spectrophotometer. The biomass that has been separated from two bottles was suspended in deionized water obtained from Thermo Branstead TII (Type II water system) and used for characterization by pipetting 4ml of each in quartzes cuvette and scanned by UV/VIS Spectrometer [27].

Transmission electron microscopy

Before and after the treatment with Pb(NO3)2, cells of isolate were examined by TEM using by JEOL JEM 2010 FEF (UHR) electron microscopy in order to identify the location of lead particles within the cells. 2µl aqueous suspensions of biomass from each two bottles were pipetted onto 400 mesh carbon coated copper grids. Samples allowed drying and evaporating in hood. TEM images were collected with JEOL JEM 2010 FEF (UHR) electron microscopy [28].

Dynamic light scattering (D.L.S)

The synthesized nanoparticles size was measured using Marlvern Zetasizer to determine the particles size. The biomass that has been separated from two bottles was suspended in deionized water obtained from Thermo Branstead TII (Type II water system) and sonicated for 10 min, and used for size characterization by pipetting 1.5 ml was put in quartz cuvette and measured in Zetasizer Nano-ZS90 (Malvern Instruments) [29].

X-ray powder diffraction analysis (XRD)

XRD was performed to detect any change in the form of Pb (NO3)2 by a transformation process as a result of an oxidation–reduction reaction occurring in both isolates After treating with Pb, cells of both isolates were oven-dried at 50°C and then kept in sterilized small glass vials and examined using an XRD.

The crystalline structures of the prepared powders were analyzed by X-ray diffractometric (XRD) (X'Pert PRO, PANalytical, Netherlands) using Cu Kα radiation in the angular region of $2\theta = 4^{o}$-70^{o}. The instrument was operated at 40KV and the spectra were recorded at scanning speed of 8^{o}/min [30].

Results

Physico-chemical Characteristics of Waste Water

Three sampling runs were carried out to monitor the variation in the physico-chemical parameters in the three main sites of the wastewater plant (influent, 2ry sedimentation tank and effluent). Results of physico-chemical parameters (pH, total dissolved solid (TDS), electrical conductivity (EC), total suspended solids (TSS), chemical oxygen demand (COD), biochemical oxygen demand (BOD_5), ammonia nitrogen (NH_3), phosphate, oil and grease and heavy metal) were presented in Table (1).

Microbiological analysis result of wastewater:

Eumeration of wastewater bacteria:

The bacterial count of wastewater samples (influent, 2ry sedimentation tank and effluent) which grew on TSA media and incubated for 48 h was calculated by Interscience automatic colony counter, model Scan 500, France and were presented in Table (2). The total number of bacteria was much higher in the influent site (73×10^6 CFU/ml) than in the other two sites after the biological treatment (33×10^4 CFU/ml) and after the addition of chlorine (21×10^2 CFU/ml).

Characterization of isolated bacteria (morphology & gram stain):

The bacterial colonies were isolated and purified on TSA plates by streaking to obtain pure cultures which reached to 29 different isolates. Isolates number from 1 to 13 isolated from influent samples, isolates number from 14 to 22 isolated from after 2ry sedimentation tank sample and isolates number from 23 to 29 isolated from effluent. All different isolates characterized by shape, edge, color, elevation and gram stain and these result were presented in table (3)

Selection and identification of most lead ions tolerant bacteria

All 29 isolates were inoculated by streaking on TSA medium contaminated with 10 ppm for Pb(II) (as Pb(NO3)2).

After 72h incubation period, isolates 1, 21, 23, 26 and 29 showed most tolerant to lead as shown in Fig. (3)

The most five potent isolates in lead resistant were identified by biochemical tests via strip system (BioMereux) on Vitek 2C. The most lead resistant isolates were isolated from wastewater samples were as follow:

Bacterial isolate number 1 which isolated from influent of Zenine wastewater treatment plant was *Shewanella putrefaciens*, which biochemical test results were presented in table (4).

Bacterial isolate number 21 which isolated after 2ry sedimentation tank of Zenine wastewater treatment plant was *Escherichia coli* , which biochemical test results were presented in table (5).

Bacterial isolate number 23 which isolated from effluent of Zenine wastewater treatment plant was *Escherichia coli* which biochemical test results were presented in table (6).

Bacterial isolate number 26 which isolated from effluent of Zenine wastewater treatment plant was *Acinetobacter sp*., which biochemical test results were presented in table (7).

Bacterial isolate number 29 which isolated from effluent of Zenine wastewater treatment plant was *Serratia plymuthica*, which biochemical test results were presented in table (8).

Minimum Inhibitory Concentration (MIC) for lead on Serratia plymuthica

The Minimum Inhibitory Concentration (MIC) for lead ions on *Serratia plymuthica* was 80 mg/l (ppm). The growth rate of *Serratia plymuthica on* TSA media which contaminated with different concentration of lead Pb(II) (as $Pb(NO_3)_2$) presented in Table (4).

Synthesis of Lead Nanoparticles:

After 72 hrs. of incubation a deep change in color was observed in the flask containing tryptic soy bean broth containing 40ppm from lead nitrate while there was no change in color in the other flask containing tryptic soy broth as depicted in Figure (4). This primarily indicated that nanoparticle synthesis took place in the tryptic soy bean broth media containing lead nitrate.

Characterization of Nanoparticles:

UV-Vis Spectroscopy:

The synthesized nanoparticle suspended in deionized water was scanned from 200-400nm using PerkinElmer UV/VIS Spectrometer- Lambda 25 spectrophotometer showing high absorbance at ~ 330 nm figure (5), which is a specific peak for lead nanoparticles.

Transmission electron microscopy:

High Resolution Transmission Electron Microscopy (HRTEM) was performed by JEOL JEM 2010 FEF (UHR) electron microscope with an accelerating voltage of 200 kV. Figure (5.5) shows the TEM images for pellets of *S. plymuthica* inoculated in TSB without lead nitrate and with lead nitrate respectively. The TEM image show formation of Pb NPs intracellular and extracellular and cells aggregation in case of *S. plymuthica* inoculated in TSB with lead nitrate.

$$NH_4^+ + 1.5O_2 \xrightarrow[Nitrospira\ sp]{Nitrosomonas\ sp} NO_2^- + H_2O + 2H^+$$

Dynamic light scattering (D.L.S):

Hydrodynamic size distribution of PbNPs was analyzed using dynamic light scattering. (DLS). D.L.S results show that *S. plymuthica* formation of new particles in TSB media that containing lead nitrate with average size equal to 92.93nm, where the size distribution average for deionized water (blank) was 463.57 nm and the size distribution average for TSB media that inoculating with *S.plymuthica* without lead nitrate (control) was 1440 nm as shown in figures (8), (9) and (10) and tables (10), (11) and (12).

X-ray powder diffraction analysis (XRD):

Chemical composition of PbNPs was determined using XRD analysis of dried Pb(NO3)2-treated cells of S. plymuthica approved the synthesis of lead sulphide (PbS) nanoparticles as shown in figure (11).

DISCUSSION:

The used treatment process in Zenine Wastewater Treatment Plant is the activated sludge process which is common in wastewater treatment plants and was developed before more than 100 years and is primarily used for removal of dissolved and colloidal biodegradable organic compounds by bacteria present in the sludge oxidize the organics into carbon dioxide and water and had ability to resist and bioremediation of heavy metal [31, 32]

Three sampling runs were carried out to monitor the variation in the physico-chemical parameters in the three main sites of the wastewater plant (influent, 2ry sedimentation tank and effluent). The pH was found to be around 7 which is normally optimum for activated sludge process operations, that was reported by [33].The influent site recorded high values in all tests; TSS, TDS, COD, BOD_5, Oil and grease, NH_3 and heavy metals. This is due to that the wastewater in the influent was not treated yet and the wastewater usually contains complex organic and inorganic substances. NH_3 decreased from 34 mg/l in the influent to 12 mg/l in the effluent due to nitrification. Nitrification is the oxidation of ammonium to nitrate, which was done by two groups of obligately aerobic autotrophic proteobacteria. First ammonium was oxidized to nitrite by the ammonium oxidizers as, *Nitrosomonas* sp. and *Nitrospira* sp.:

The nitrite so produced was then oxidized to nitrate by the nitrate oxidizers, *Nitrobacter* sp., with the oxygen atom added to the nitrite ion coming from water:

That was reported by [34, 35]. Other physico-chemical characteristics decreased

$$NO_2^- + H_2O \xrightarrow{Nitrobacter\ sp} NO_3^- + 2H^+$$

significantly from the influent site to the effluent before and after chorine treatment due to activated sludge treatment process, where BOD_5average decreased from 276 mg/l in influent to 40 mg/l in effluent (85.5% removal), COD average decreased from 417 mg/l in influent to 73 mg/l in effluent(82.5% removal) and TSS average decreased from 130 mg/l in influent to 10 mg/l in effluent(92.3% removal) indicating good treatment in the plant, These average of results were in agreement with[36], also heavy metals entered the plant in the influent site decreased, where Cr average decreased from 0.052 mg/l in influent to 0.004 mg/l in effluent(92.3% removal),Cu average decreased from 0.016 mg/l in influent to 0.003 mg/l in effluent(81.25% removal),Fe average decreased from 0.875 mg/l in influent to 0.197mg/l in effluent(77.5% removal), Ni average decreased from 0.004 mg/l in influent to 0.002 mg/l in effluent(50% removal) and Pb average decreased from 0.039 mg/l in influent to 0.017 mg/l in effluent(56.4% removal) as there were many microorganisms in wastewater had biosorption properties and had ability to uptake high levels of heavy metal, which these result was complied with [24].

The total number of bacteria was much higher in the influent site (73×10^6 CFU/ml) than in the other two sites after the biological treatment (33×10^4 CFU/ml) and after the addition of chlorine (21×10^2 CFU/ml). This significant decrease in the count of bacteria was due to diminution in the concentration of the organic matters which led to progressive slow-down of the growth rate and the number of bacteria, that reported by[37], also addition of chlorine decreased the count of bacteria in effluent due to its disinfectant properties. All bacterial isolates were isolated from wastewater samples were gram negative bacteria, these results complied with [38].

Contamination of natural environment by heavy metals through industrial activities has the potential to affect the health of organisms and the environment due to the toxicity of these substances and difficulty in their remediation as Lead (Pb), which requires special attention as it is a cumulative poison because of its high toxicity and long-term persistence in the environment. It has been well documented that prolonged exposure in humans to lead can cause dangerous effects as anemia, reproductive impairment, renal failure, and neurodegenerative damage as

reported by [39]**.** Several microorganisms were reported to tolerate toxic concentration of heavy metals [40]**.** So we isolated and identified the most lead resistant bacterial isolates.

The most lead resistant isolates were isolated from wastewater samples were*Shewanella putrefaciens*, 2 isolates from *Escherichia coli*, *Acinetobacter sp.*and *Serratia plymuthica*. These results were complied with [7]**,** which ability of *Shewanella putrefaciens, E. coli*and*Acinetobacter sp.*to resist lead was reported by them.

Serratia plymuthica was selected for determination of Minimum Inhibitory Concentration (MIC) for lead and synthesis of lead nanoparticle due to the poor information about this organism. *Serratia plymuthica* showed Minimum Inhibitory Concentration (MIC) for lead at 40 ppm.

Serratia plymuthica was used to biosynthesis of lead nanoparticle (PbNPs) through inoculating it in TSB medium containing 40 ppm from $Pb(No_3)_2$which show dark color when compared with control, which containing inoculum from *Serratia plymuthica* in TSB medium. This dark color gave indication of forming PbNPs that was in agreement with [11].

Characterization of PbNPs was obtained using UV-Vis Spectroscopy which showed sharp Plasmon peak at ~ 330 nm which this wave length was the specific for lead nanoparticles PbNPs, which reported [11, 41].

TEM showed the aggregation of bacterial cells in case of exposure to lead nitrate and ability of *S. plymuthica* to biosynthesis PbNPs intracellular through the bacterial cells. TEM showed ability of *S. plymuthica* to precipitate PbNPs inside the cell.

Characterization of PbNPs particle size was done using DLS analysis showed that the average hydrodynamic size (diameter) of the particles in case of precipitate taken from TSB media which inoculating with *S. plymuthica* was 1.258µm with intensity 100%. This average hydrodynamic size (diameter) changed in case of precipitate taken from TSB media containing $Pb(No_3)_2$ which inoculating with *S. plymuthica,* which became 92.93 nm with intensity 90.3%. These results gave us indication of formation of PbNPs by *S. plymuthica.*

XRD analysis of dried $Pb(NO_3)_2$treated cells of *S. plymuthica* indicated the synthesis of lead sulfide (PbS) nanoparticles by *S. plymuthica.* Biosynthesis of (PbS) by *S. Plymuthica* might be occur due to usage of bacteria to anglesite($PbSO_4$) as the electron acceptor and reduced this compound to poorly soluble galena (PbS), which this hypothesis was reported by [7].

All these results gave us indication about the ability of *S. plymuthica*to biosynthesis PbS nanoparticle, which has economical value as semiconductor or in solar cells manufacturing.

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TABLES

Table 1.Physico-chemical characteristics of Zenine wastewater treatment

Parameter	*Sampling sites*		
	Influent	*Sedimentation tank*	*Effluent*
PH	*7.9*	*7.6*	*7.5*
Electrical conductivity	*1025*	*996*	*968*
TDS (mg/l)	*615*	*598*	*581*
TSS (mg/l)	*130*	*21*	*10*
COD (mg/l)	*417*	*98*	*73*
BOD_5 (mg/l)	*276*	*53*	*40*
NH_3 (mg/l)	*34*	*27*	*12*
Phosphate mg/l	*6.3*	*3.7*	*3.7*
Oil and grease (mg/l)	*44*	*8*	*6*
Cd (ppm)	*0.000*	*0*	*0*
Cr (ppm)	*0.052*	*0.004*	*0.004*
Cu (ppm)	*0.016*	*0.003*	*0.003*
Fe (ppm)	*0.875*	*0.197*	*0.197*
Ni (ppm)	*0.004*	*0.002*	*0.002*
Pb (ppm)	*0.039*	*0.017*	*0.017*

Table 2Total bacterial count result for wastewater samples collected from Zenine wastewater treatment plant

Parameters	*Sampling sites*		
	Influent	*Sedimentation tank*	*Effluent*
Total bacterial count (cfu/ml)	73×10^6	33×10^4	21×10^2

Table3 Characterization of different bacterial isolates according to shape, edge, color, elevation and gram stain

Isolate no.	**Shape**	**Edge**	**Color**	**Elevation**	**Gram stain**
1	Circular	Entire	Light pink	Convex	-ve (Rods)
2	Irregular	undulate	Pale yellow	Raised	-ve (Rods)
3	Circular	Entire	White	Flat	-ve (Rods)
4	Circular	Entire	Creamy	Raised	-ve (Cocci)
5	Circular	Entire	Orange	Convex	-ve (Rods)
6	Circular	Entire	Pale yellow	Flat	-ve (Cocci)
7	Circular	Entire	yellow	Flat	-ve (Rods)
8	Circular	Entire	Creamy	Raised	-ve (Rods)
9	Circular	Entire	Colorless	Raised	-ve (Cocci)
10	Irregular	undulate	Creamy	Flat	-ve (Cocci)
11	Circular	Entire	yellow	Raised	-ve (Cocci)
12	Circular	Entire	Colorless	Flat	-ve (Rods)
13	Circular	Entire	White	Convex	-ve (Rods)
14	Irregular	Entire	Pale yellow	Flat	-ve (Coccobacilli)
15	Circular	Entire	White	Raised	-ve (Rods)
16	Circular	Entire	Creamy	Raised	-ve (Rods)
17	Circular	Entire	Colorless	Raised	-ve (Cocci)
18	Irregular	undulate	Pale yellow	Flat	-ve (Rods)
19	Circular	Entire	White	Raised	-ve (Cocci)
20	Circular	Entire	White	Convex	-ve (Rods)
21	Circular	Entire	Colorless	Raised	-ve (Rods)
22	Circular	Entire	Creamy	Raised	-ve (Cocci)
23	Circular	Entire	Colorless	Raised	-ve (Rods)
24	Circular	Entire	Pale yellow	Flat	-ve (Rods)
25	Circular	Entire	yellow	Convex	-ve (Cocci)
26	Circular	Entire	Pale yellow	Raised	-ve (Coccobacilli)
27	Irregular	undulate	White	Convex	-ve (Rods)
28	Circular	Entire	yellow	Flat	-ve (Cocci)
29	Circular	Entire	Orange	Raised	-ve (Rods)

Table 4 Identification information of isolates no 1

Biochemical Details																				
2	APPA	+	3	ADO	-	4	PyrA	+	5	IARL	-	7	dCEL	-	9	BGAL	-			
10	H2S	+	11	BNAG	+	12	AGLTp	+	13	dGLU	-	14	GGT	+	15	OFF	-			
17	BGLU	-	18	dMAL	+	19	dMAN	-	20	dMNE	-	21	BXYL	-	22	BAIap	-			
23	ProA	+	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	-			
33	SAC	+	34	dTAG	-	35	dTRE	-	36	CIT	-	37	MNT	-	39	5KG	-			
40	ILATk	+	41	AGLU	+	42	SUCT	+	43	NAGA	-	44	AGAL	-	45	PHOS	+			
46	GlyA	-	47	ODC	-	48	LDC	-	53	IHISa	-	56	CMT	-	57	BGUR	-			
58	O129R	-	59	GGAA	+	61	IMLTa	-	62	ELLM	-	64	ILATa	-						

Table 5 Identification information of isolates no 21

Biochemical Details																				
2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	+			
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	+			
17	BGLU	-	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAIap	-			
23	ProA	-	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	+			
33	SAC	-	34	dTAG	-	35	dTRE	+	36	CIT	-	37	MNT	-	39	5KG	-			
40	ILATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	+	45	PHOS	-			
46	GlyA	-	47	ODC	+	48	LDC	+	53	IHISa	-	56	CMT	+	57	BGUR	+			
58	O129R	+	59	GGAA	-	61	IMLTa	-	62	ELLM	+	64	ILATa	-						

Table 6 Identification information of isolates no 23

Biochemical Details																				
2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	+			
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	+			
17	BGLU	-	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAIap	-			
23	ProA	-	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	+			
33	SAC	-	34	dTAG	-	35	dTRE	+	36	CIT	-	37	MNT	-	39	5KG	-			
40	ILATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	+	45	PHOS	-			
46	GlyA	-	47	ODC	+	48	LDC	+	53	IHISa	-	56	CMT	+	57	BGUR	+			
58	O129R	+	59	GGAA	-	61	IMLTa	-	62	ELLM	+	64	ILATa	-						

Table 7 Identification information of isolates no 26

Biochemical Details																				
2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	+	9	BGAL	-			
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	-			
17	BGLU	-	18	dMAL	-	19	dMAN	-	20	dMNE	+	21	BXYL	-	22	BAIap	-			
23	ProA	-	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	+	32	dSOR	-			
33	SAC	-	34	dTAG	-	35	dTRE	-	36	CIT	+	37	MNT	+	39	5KG	-			
40	ILATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	-	45	PHOS	-			
46	GlyA	-	47	ODC	-	48	LDC	-	53	IHISa	-	56	CMT	+	57	BGUR	-			
58	O129R	+	59	GGAA	-	61	IMLTa	-	62	ELLM	-	64	ILATa	-						

Table 8 Identification information of isolates no 29

Biochemical Details																				
2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL		9	BGAL	-			
10	H2S	-	11	BNAG	+	12	AGLTp	-	13	dGLU	+	14	GGT		15	OFF	-			
17	BGLU	+	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL		22	BAIap	-			
23	ProA	-	26	LIP	-	27	PLE	+	29	TyrA	-	31	URE		32	dSOR	+			
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	-	37	MNT		39	5KG	-			
40	ILATk	+	41	AGLU	+	42	SUCT	-	43	NAGA	-	44	AGAL		45	PHOS	+			
46	GlyA	-	47	ODC	-	48	LDC	-	53	IHISa	-	56	CMT		57	BGUR	-			
58	O129R	+	59	GGAA	-	61	IMLTa	-	62	ELLM	-	64	ILATa							

Table 9 Growth rate of serratia plymuthica on TSA media contaminated with Pb(II)

Lead concentration in mg/L	10	20	30	40	50	60	70	80	90
Growth of Serratia plymuthica	+++	+++	++	++	++	+	+	+	-

Table 10 Size Statistics table by Number for deionized water (Blank)

Size d.nm	Mean number %	Size d.nm	Mean number %	Size d.nm	Mean number %	Size d.nm	Mean number %
0.4000	0.0	5.615	0.0	78.82	0.0	1106	0.0
0.4632	0.0	6.503	0.0	91.28	0.0	1281	0.0
0.5365	0.0	7.531	0.0	105.7	0.0	1484	0.0
0.6213	0.0	8.721	0.0	122.4	0.0	1718	0.0
0.7195	0.0	10.10	0.0	141.8	0.0	1990	0.0
0.8332	0.0	11.70	0.0	164.2	0.0	2305	0.0
0.9649	0.0	13.54	0.0	190.1	3.4	2669	0.0
1.117	0.0	15.69	0.0	220.2	14.4	3091	0.0
1.294	0.0	18.17	0.0	255.0	26.3	3580	0.0
1.499	0.0	21.04	0.0	295.3	27.6	4145	0.0
1.736	0.0	24.36	0.0	342.0	18.8	4801	0.0
2.010	0.0	28.21	0.0	396.1	7.9	5560	0.0
2.328	0.0	32.67	0.0	458.7	1.6	6439	0.0
2.696	0.0	37.84	0.0	531.2	0.0	7456	0.0
3.122	0.0	43.82	0.0	615.1	0.0	8635	0.0
3.615	0.0	50.75	0.0	712.4	0.0	1.000e4	0.0
4.187	0.0	58.77	0.0	825.0	0.0		
4.849	0.0	68.06	0.0	955.4	0.0		

Table 11 Size Statistics by Number for yielded S. plymuthica pellet in TSB media without lead nitrate which suspended in deionized water (Control)

Size d.nm	Mean number %	Size d.nm	Mean number %	Size d.nm	Mean number %	Size d.nm	Mean number %
0.4000	0.0	5.615	0.0	78.82	0.0	1106	19.7
0.4632	0.0	6.503	0.0	91.28	0.0	1281	23.7
0.5365	0.0	7.531	0.0	105.7	0.0	1484	20.0
0.6213	0.0	8.721	0.0	122.4	0.0	1718	8.8
0.7195	0.0	10.10	0.0	141.8	0.0	1990	1.8
0.8332	0.0	11.70	0.0	164.2	0.0	2305	0.7
0.9649	0.0	13.54	0.0	190.1	0.0	2669	0.3
1.117	0.0	15.69	0.0	220.2	0.0	3091	0.2
1.294	0.0	18.17	0.0	255.0	0.0	3580	0.1
1.499	0.0	21.04	0.0	295.3	0.0	4145	0.0
1.736	0.0	24.36	0.0	342.0	0.0	4801	0.0
2.010	0.0	28.21	0.0	396.1	0.0	5560	0.0
2.328	0.0	32.67	0.0	458.7	0.0	6439	0.0
2.696	0.0	37.84	0.0	531.2	0.0	7456	0.0
3.122	0.0	43.82	0.0	615.1	0.5	8635	0.0
3.615	0.0	50.75	0.0	712.4	2.9	1.000e4	0.0
4.187	0.0	58.77	0.0	825.0	7.6		
4.849	0.0	68.06	0.0	955.4	13.8		

Table 12 Size Statistics by Number for yielded S. plymuthica pellet in TSB media with lead nitrate which suspended in deionized water (Test)

Size d.nm	Mean Number %	Size d.nm	Mean Number %	Size d.nm	Mean Number %	Size d.nm	Mean Number %
0.4000	0.0	5.615	0.0	78.82	23.0	1106	0.6
0.4632	0.0	6.503	0.0	91.28	23.6	1281	0.6
0.5365	0.0	7.531	0.0	105.7	16.0	1484	0.4
0.6213	0.0	8.721	0.0	122.4	8.3	1718	0.2
0.7195	0.0	10.10	0.0	141.8	3.4	1990	0.0
0.8332	0.0	11.70	0.0	164.2	1.1	2305	0.0
0.9649	0.0	13.54	0.0	190.1	0.4	2669	0.0
1.117	0.0	15.69	0.0	220.2	0.3	3091	0.0
1.294	0.0	18.17	0.0	255.0	0.4	3580	0.0
1.499	0.0	21.04	0.0	295.3	0.6	4145	0.0
1.736	0.0	24.36	0.0	342.0	0.8	4801	0.0
2.010	0.0	28.21	0.0	396.1	0.8	5560	0.0
2.328	0.0	32.67	0.0	458.7	0.8	6439	0.0
2.696	0.0	37.84	0.0	531.2	0.7	7456	0.0
3.122	0.0	43.82	0.0	615.1	0.7	8635	0.0
3.615	0.0	50.75	0.0	712.4	0.7	1.000e4	0.0
4.187	0.0	58.77	2.7	825.0	0.7		
4.849	0.0	68.06	12.4	955.4	0.7		

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FIGURES

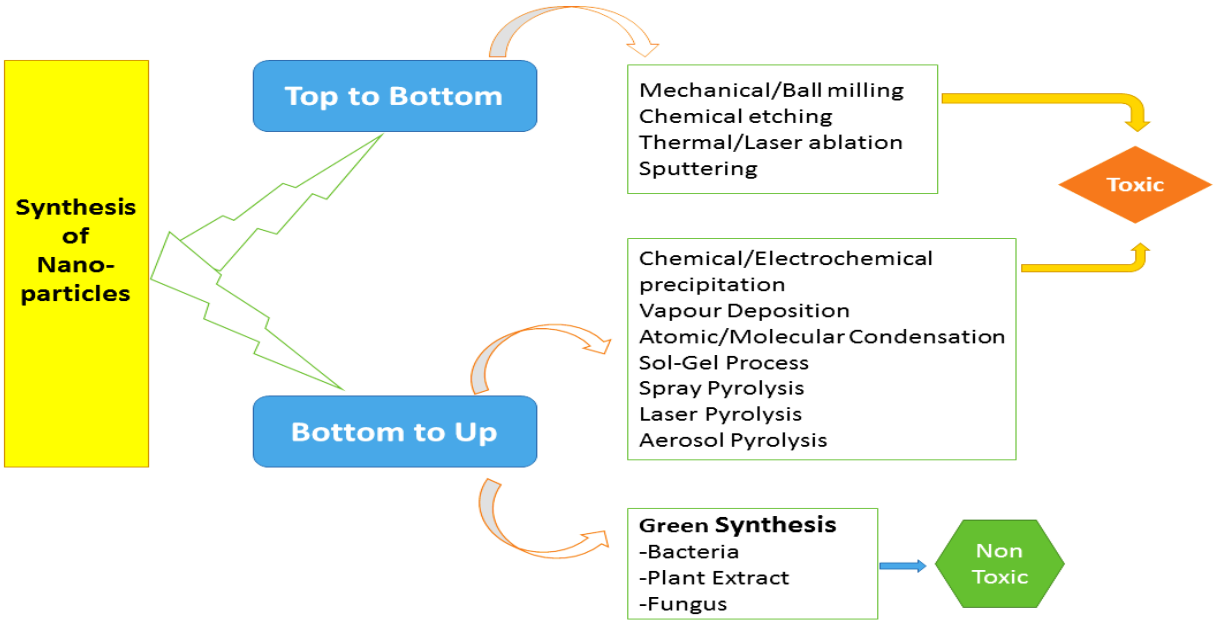

Figure 1 Different approaches of synthesis of silver nanoparticles [13].

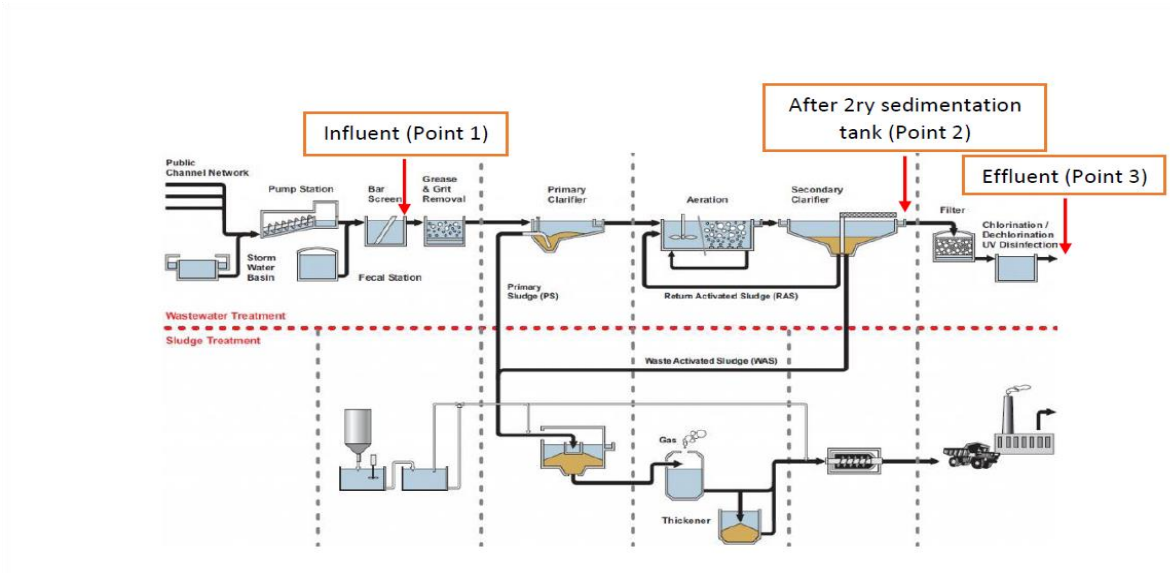

Figure 2 Scheme illustrate the sampling points was taken from Zenine wastewater treatment plant [19]

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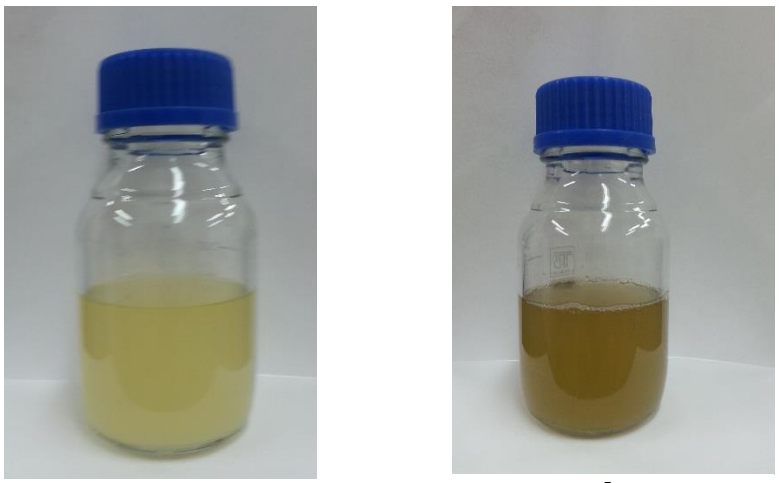
Figure 3 the most lead tolerant bacteria isolates (1, 21, 23, 26, 29)



a **b**

Figure 4 (a) Tryptic soy bean broth media inoculated with S.plymutica after 72h incubation period, (b) Tryptic soy bean broth media containing 40 ppm (Pb(No3)2) inoculated with S.plymutica after 72h incubation period

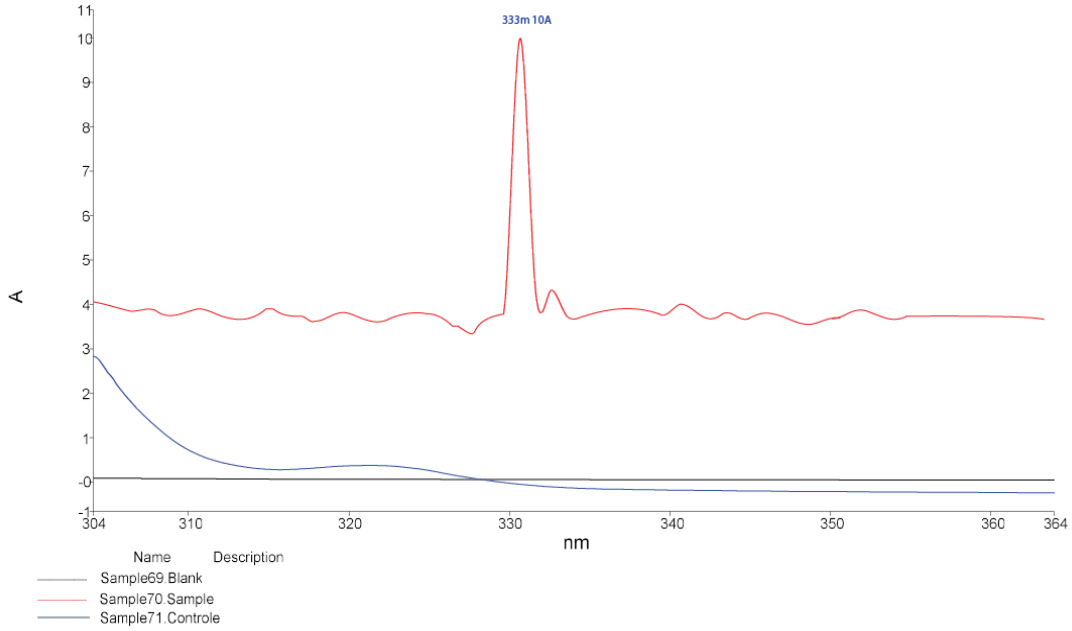


Figure 5 UV-Vis absorbance Maxima of PbNPs after 72h of incubation. Red line refers to sample (Synthesized particle suspended in deionized water), Blue line refers to control pellets of bacterial suspend in deionized water, Black line refer to deionized

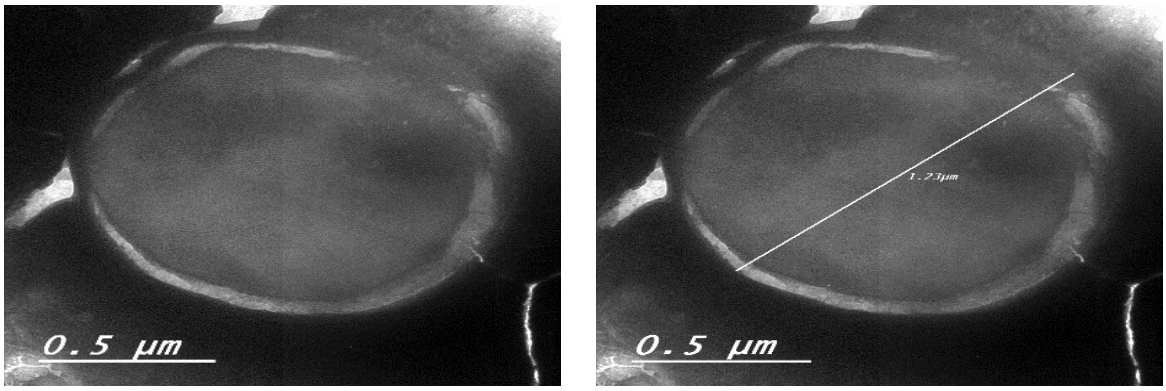


Figure 6 (a) Transmission electron microscope image of S.plymutica inoculated in TSB without lead nitrate

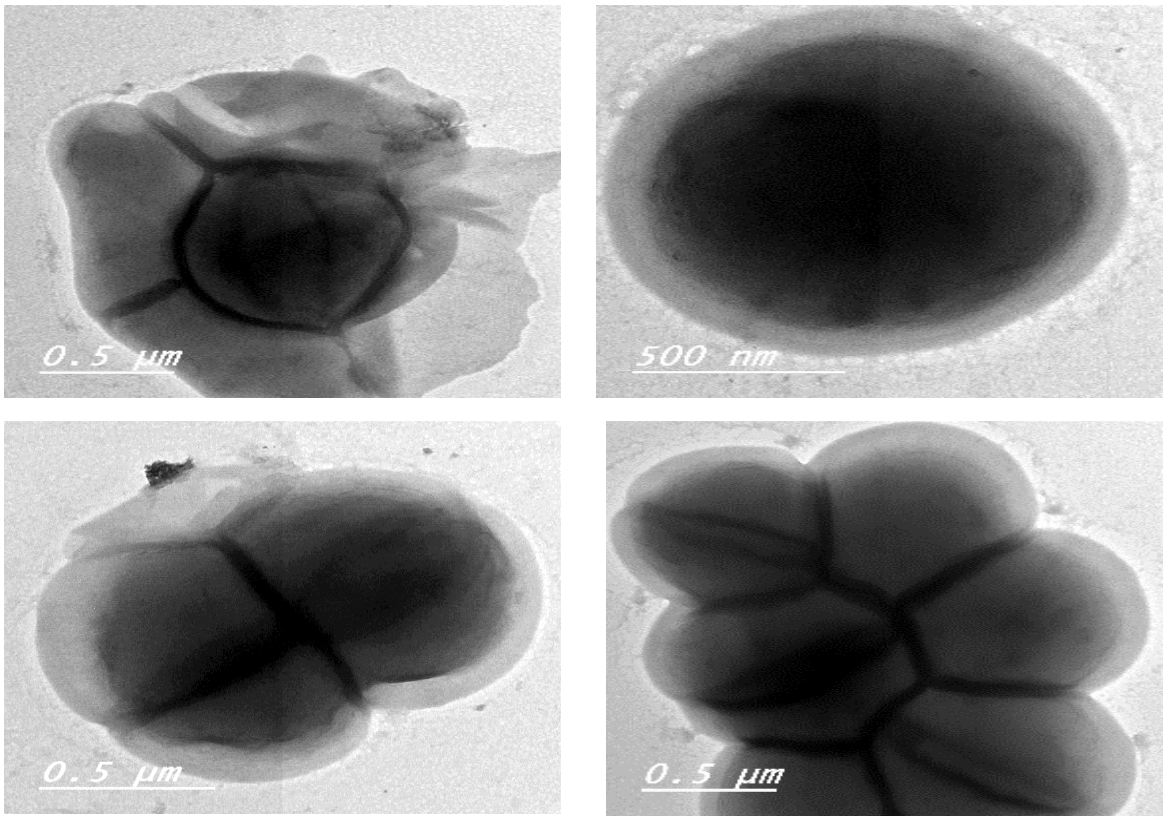


Figure 7 (b) Transmission electron microscope image of S. plymuthica inoculated in TSB containing lead nitrate

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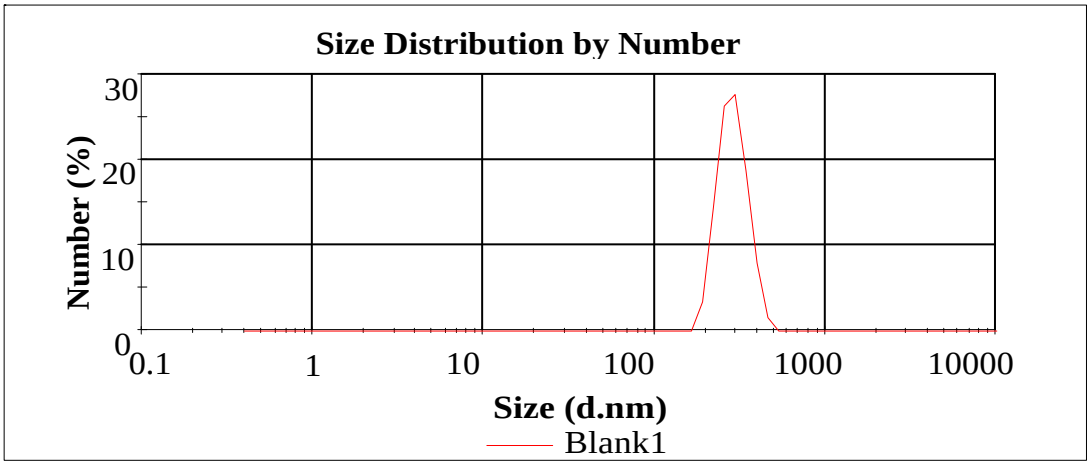

Figure 8 (a) Size Distribution chart by Number for deionized water (Blank)

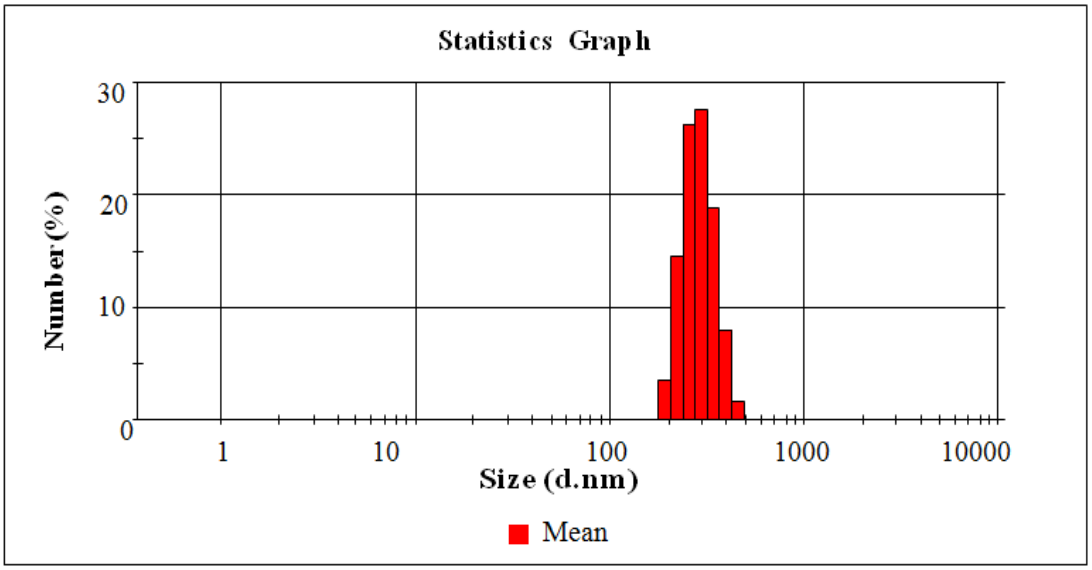

Figure 8 (b) Size Statistics graph by Number for deionized water (Blank)

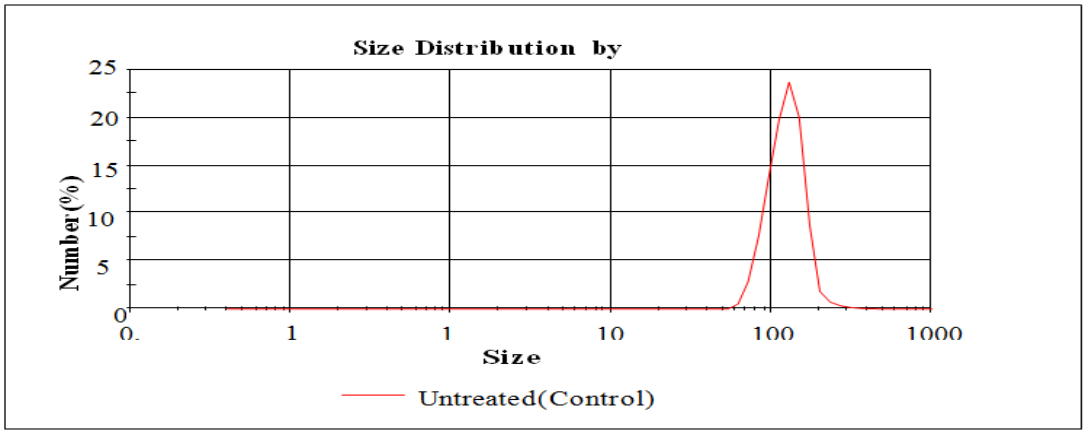

Figure 9 (a) Size Distribution chart by Number for yielded S. plymuthica pellet in TSB media without lead nitrate which suspended in deionized water (Control)

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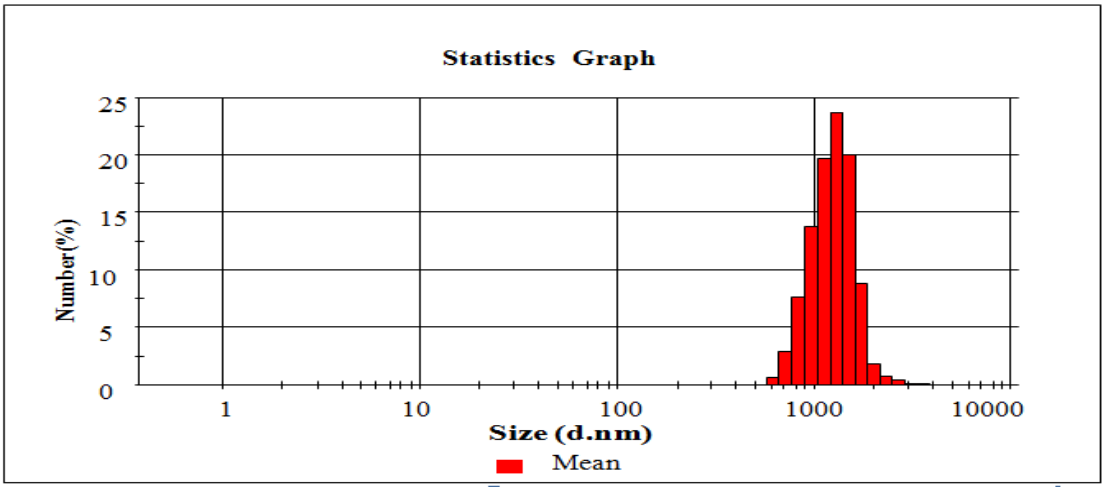


Figure 9 (b) Size Statistics graph by Number for yielded S. plymuthica pellet in TSB media without lead nitrate which suspended in deionized water (Control)

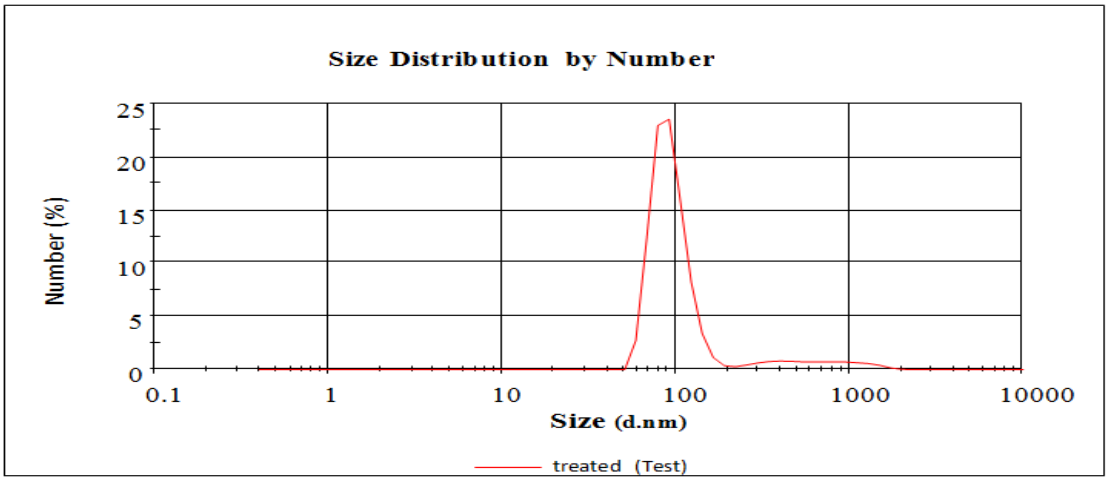


Figure 10(a) Size Distribution chart by Number for yielded S. plymuthica pellet in TSB media with lead nitrate which suspended in deionized water (Test)

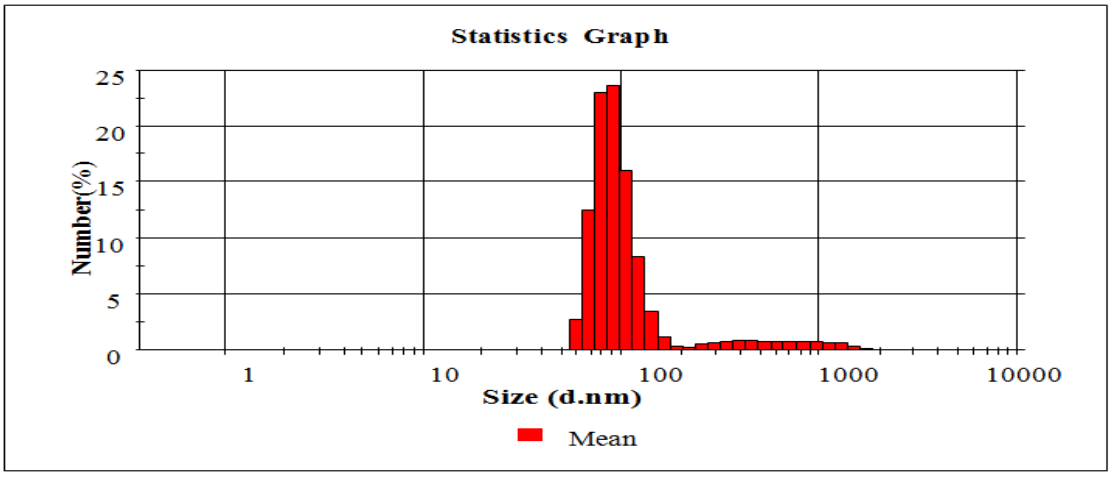
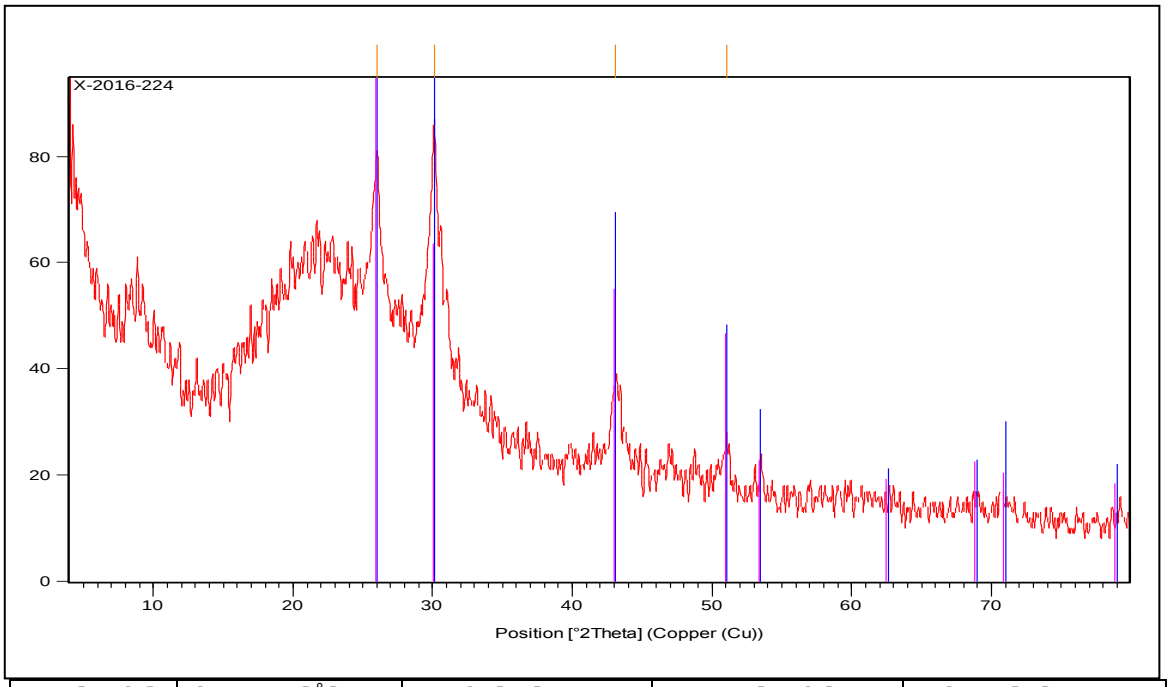


Figure 10 (b) Size Statistics graph by Number yielded S. plymuthica pellet in TSB media with lead nitrate which suspended in deionized water (Test)



Pos. [°2Th.]	d-spacing [Å]	Height [cts]	FWHM [°2Th.]	Rel. Int. [%]
30.1174	2.96732	43.21	0.3149	100.00
Visible	Ref. Code	Chemical Formula	PDF Index Name	Mineral Name
Blue line	03-066-0020	Pb S	Lead Sulfide	Galena, syn

Figure 11 X-ray diffract gram of PbS nanoparticles synthesized by S. plymuthica.