

## Chapter 5

# Extraction of Silver Nanoparticles (Ag-NPs) by Green Synthesis from Aqueous Extract of Seaweeds and Their Consequences on HeLa Cell Line and Their Utility on Soil by Spectroscopic Tools



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**Abstract** Seaweed extracts have been used to synthesize silver nanoparticles (Ag-NPs) as they offer many benefits and utilities. The use of biological materials in nanoparticles synthesis is preferred as they are eco-friendly and compatible for pharmaceutical and other biomedical and therapeutic applications. As the number of applications of NPs increases, assessment of the risks posed by NPs is important and it has developed into an area of research itself. Metallic nanoparticles have different applications such as in electronics, catalysts and photonic applications. Silver metal has toxicity against a wide range of micro organisms especially silver nanoparticles which has promising antimicrobial properties and other bio utilities. Silver nanoparticles are effective as anti-inflammatory, anti-angiogenesis, antiviral and

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anti-platelet activity against cancer cells. The Ag-NPs synthesized were from the aqueous extracts of seaweeds and were characterized by using spectral analysis as a perspective tool. The effect of the synthesized silver nanoparticles on the isolated soil microbes were assessed by different assays such as Calorimetric chowder assay and Agar diffusion assay. The effect of synthesized Ag-NPs on earthworm was also studied by examining its morphology and histology. The toxicity of the combined seaweed-AgNPs against HeLa carcinoma cells has been analyzed. Also the Ag-NPs are studied with Fluorescence—FL activity with band gap in eV.

**Keywords** Silver NPs · Green synthesis · Seaweed · HeLa cell line · Soil · Spectroscopic tools

## 5.1 Synthesis and Characterization of Silver Nanoparticles

Silver nanoparticles have been broadly utilized for the time of the point of reference hardly many years in different applications because of their notable adequacy in biomedical (Cao et al. 2010), electronic (Mohan et al. 2007), catalysis (Feng et al. 2011) and optical applications (Hayward et al. 2000). Specifically, the extraordinary antimicrobial properties of Ag-NPs have prompted the advancement of a wide assortment of nano silver things, including nanosilver-covered injury dressings, prophylactic gadgets, vigilant instruments, and embeds (Lohse and Murphy 2012; You et al. 2012; Aziz et al. 2016). Aside from these antimicrobial exercises, Ag-NPs are likewise known to have antifungal, calming, antiviral, hostile to angiogenesis and against platelet properties. Also, later improvements have seen Ag-NPs utilized in room shower, backdrop gloves, clothing cleanser, and divider paint definitions just as in the material business for garments producing. The current examination depicts a solitary advance, green, and fast combination of silver nanoparticles (Ag-NPs) arranged by organic (green) strategies utilizing kelp concentrates of *Rhodymenia palmata*, *Gracilaria corticata*, *Hypnea musciformis*, *Sargassum tenerrimum*, *Stoechospermum marginatum* and *Dictyota dichotoma*.

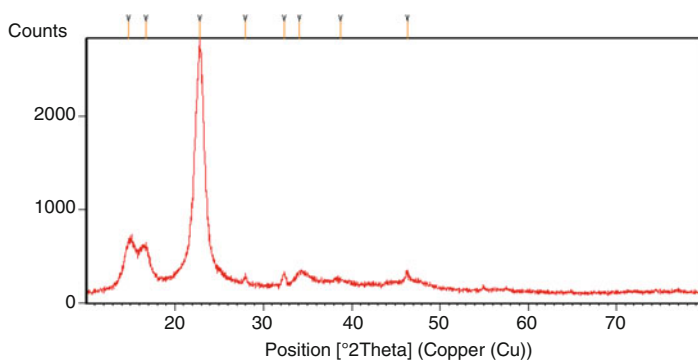
Watery arrangement of 1 mM silver presented to fluid marine growth concentrates of *Rhodymenia palmata*, *Gracilaria corticata*, *Hypnea musciformis*, *Sargassum tenerrimum*, *Stoechospermum marginatum* and *Dictyota dichotoma* a noticeable shading change from straightforward, light earthy colored to dim earthy colored in 5 minutes at room temperature. It indicates the pattern of silver nanoparticles which was confirmed by spectral analysis. The amalgamations of silver nanoparticles were observed at regular intervals through UV visible spectroscopy. The shading change deduction is one of least demanding realized procedures to affirm the nanoparticles union as there is decline in size with an expansion in excitation of external surface electrons known as surface plasmon reverberation (Ding et al. 2015).

The movement of the response prompting the change of  $\text{Ag}^+$  from  $\text{AgNO}_3$  to decreased nano one by watery concentrate of *Rhp* (*Rhodymenia palmata*), *Grc*

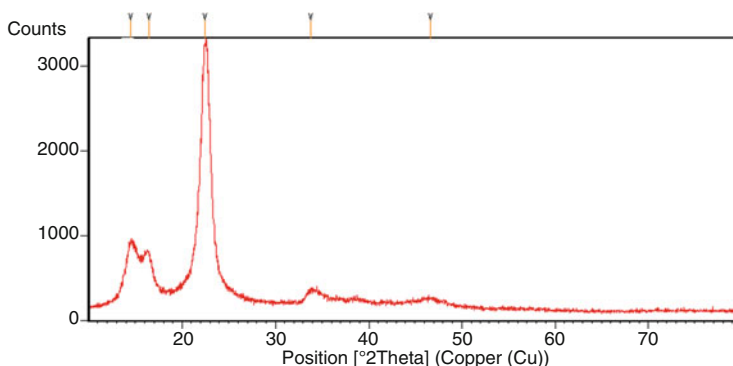
(*Gracilaria corticata*), *Hym* (*Hypnea musciformis*), *Srt* (*Sargassum tenerrimum*), *Stm* (*Stoechospermum marginatum*) and *Dtd* (*Dictyota dichotoma*). Silver nanoparticles were checked by watching the shading change and absorbance maxima top in the scope of around 416 nm. The apex showed a surface plasmon reverberation (SPR), which has just been recorded for different metal nanoparticles which went from 2 to 100 nm in size (Henglein 1993; Ravindra and Rajasab 2014). The state of the band was even proposing uniform dispersal of nanoparticles (Travan et al. 2009). Absorbance maxima pinnacle of *Grc*-AgNPs, *Hym*-AgNPs (*Gracilaria corticata*, *Hypnea musciformis* ocean growth blended silver nanoparticles) is in the scope of 375 nm affirming the development of silver nanoparticle (Baia and Simon 2007; Ayman et al. 2014). The recurrence and width of the surface plasmon assimilation relies upon the size and state of the metal nanoparticles just as on the dielectric steadiness of the metal itself and the encompassing medium (Mukherjee et al. 2002).

## 5.2 XRD

X-beam or X-ray diffraction (XRD) of silver nanoparticles delivered utilizing *Gracilaria corticata* and *Hypnea musciformis* are appeared in the Figs. 5.1 and 5.2. Various Bragg's reflections with  $2\theta$  estimations of 38, 44, 6 and 77 which coordinate to the (111), (200), (220) and (311) arrangements of grid planes are experiential which are recorded to the face-focused cubic structures for silver. The widened Bragg's pinnacle shows the development of nano crystals (Theivasanthi and Alagar 2010; Zargar et al. 2011). The XRD pattern of the pinnacles showed that the silver nanoparticles blended by *Gracilaria corticata* and *Hypnea musciformis* are translucent in nature and a portion of the unassigned acmes were additionally watched, it might be expected to the less bimolecular of settling operators, catalysts or proteins in the marine growth.



**Fig. 5.1** XRD diffraction patterns recorded from drop coated films of silver nanoparticles on *Grc*-AgNPs

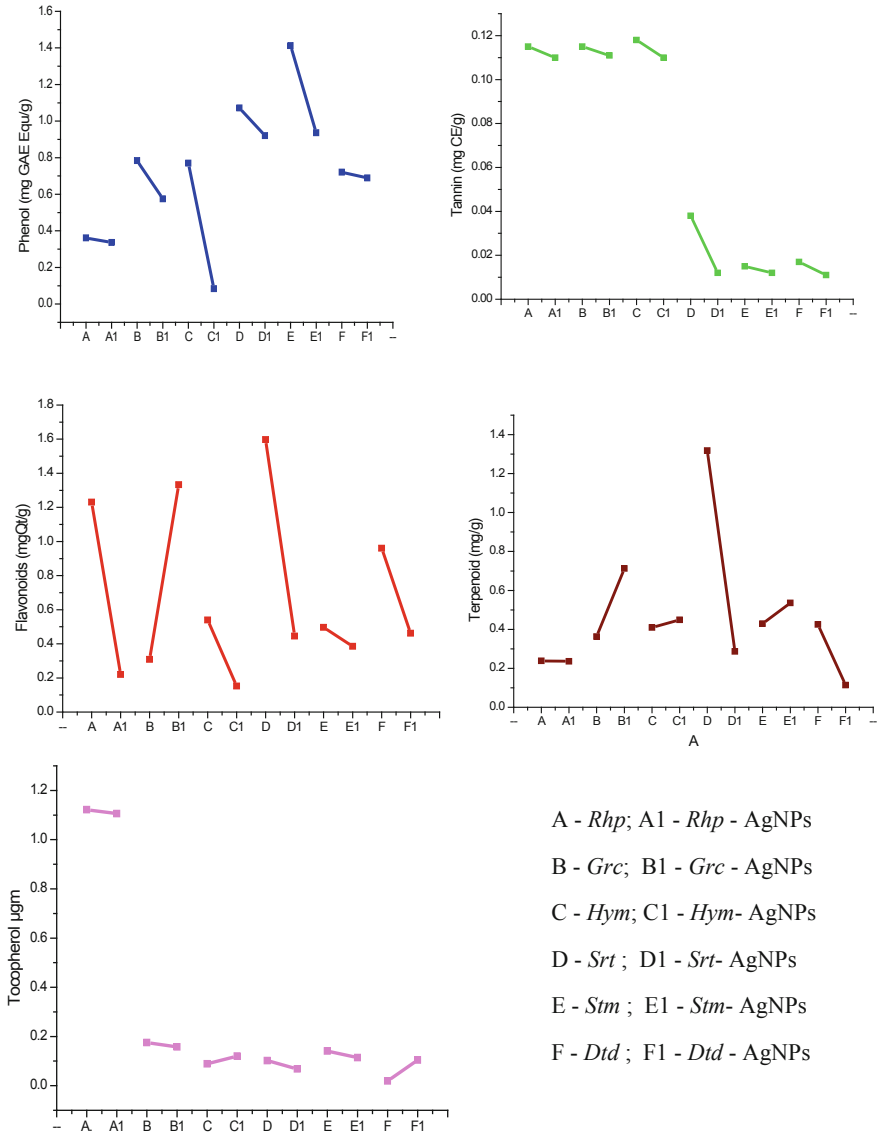


**Fig. 5.2** XRD diffraction patterns recorded from drop coated films of silver nanoparticles on *Hym-AgNPs*. *Grc-AgNPs-Gracilaria corticata-AgNPs*. *Hym-AgNPs—Hypnea musciformis-AgNPs*

### 5.3 Antioxidant

Antioxidant association of thorough going phenols, tannins, flavonoids, tocopherol and terpenoids contents of watery concentrates of *Rhodymenia palmata*, *Gracilaria corticata*, *Hypnea musciformis*, *Sargassum tenerrimum*, *Stoechospermum marginatum* and *Dictyota dichotoma*, *Rhp-AgNP* (Silver nano incorporated by *Rhp*), *Grc-AgNP* (Silver nano orchestrated by *Grc*) and *Hym-AgNP* (Silver nano combined by *Hym*), *Srt-AgNP* (Silver nano integrated by *Srt*), *Stm-AgNP* (Silver nano blended by *Stm*) and *Dtd-AgNP* (Silver nano combined by *Dtd*) introduced, showed in Fig. 5.3 that these synthetic substance were prevalently higher in fluid concentrate of *Rhodymenia palmata*, *Gracilaria corticata*, *Hypnea musciformis*, *Sargassum tenerrimum*, *Stoechospermum marginatum* and *Dictyota dichotoma* contrasted with the ocean growth diminished *AgNPs* (*Rhp-AgNPs*, *Grc-AgNPs*, *Hym-AgNPs*, *Srt-AgNPs*, *Stm-AgNPs* and *Dtd-AgNPs*). Yet, terpenoid substance of *Grc-AgNPs* and *Rhp-AgNPs* tocopherol content were higher than the fluid concentrate.

In the current examination, cancer prevention agent limit saw on the nearness of all out phenol, tannin, flavonoid, tocopherol and terpenoid content. The significant measure of cancer prevention agents in the watery concentrate of *Rhodymenia palmata*, *Gracilaria corticata*, *Hypnea musciformis*, *Sargassum tenerrimum*, *Stoechospermum marginatum* and *Dictyota dichotoma* were noted. Polyphenolic mixes are normal cell reinforcements which are found for the most part in kelp (Moon and Shibamoto 2009). Polyphenolics contain lessening properties as hydrogen or electron giving specialists, in this way observed as cell reinforcements. So our outcome uncovered that the concentrate of marine kelp *Rhodymenia palmata*, *Gracilaria corticata*, *Hypnea musciformis*, *Sargassum tenerrimum*, *Stoechospermum marginatum* and *Dictyota dichotoma*, were fit for creating *Ag* nanoparticles extracellular and these nano particles are very steady in arrangement due to topping likely by the polyphenols present in the concentrate.



**Fig. 5.3** Comparison of amount of antioxidant contents in seaweeds and seaweed synthesised silver nanoparticles

## 5.4 In-Vitro Cytotoxicity Investigation of Hym—AgNPs on HeLa Cell Line

Nanoparticles (NPs) of various size and physicochemical properties have been acquainted with numerous fields of life and biomedical sciences in the course of the most recent decade (Oberdorster et al. 2005). In this regard, NPs opened another period in biomedical sciences and have been utilized explicitly as quality or operator bearers, and in sedate plan, change of therapeutics, naming of fluorescents, and tissue building (Tan et al. 2007; Yoon et al. 2007; Kreuter and Gelperina 2008; Su et al. 2008; Hackenberg et al. 2010). Among the NPs, silver nanoparticles (AgNPs) have gotten consideration for their antimicrobial exercises (Cho et al. 2005; Kim et al. 2006; Prasad et al. 2011, 2012; Prasad and Swamy 2013; Prasad 2014; Joshi et al. 2018) and have been utilized for various purposes including the assembling of disinfectants, shampoos, antiperspirants, humidifiers, wound dressings, and different material items (Ahmed et al. 2008; Johnston et al. 2010; Zanette et al. 2011) they have likewise been utilized as a covering for different implantable gadgets, for example, catheters, heart valves, and inserts (Chen and Schluesener 2008; Chaloupka et al. 2010). Regardless of their advantages, there has been not kidding worry about the conceivable symptoms of AgNPs. Past investigations detailed that AgNPs actuated genotoxicity and cytotoxicity in both malignant growth and typical cell lines (Yoon et al. 2007; Aziz et al. 2019), adjusted cell morphology, decreased cell suitability, and caused oxidative worry in lung fibroblast and glioblastoma cells (Asharani et al. 2009), human and rodent liver cells (Hussain et al. 2005; Kim et al. 2010), HeLa cells (Sonoda et al. 1998) and THP-1 monocytes (Foldbjerg et al. 2009). AgNP-initiated cytotoxicity decreases cell feasibility in different cell lines by causing apoptosis through the mitochondrial pathway (Hsin et al. 2008) and creates oxidant species (Hess et al. 2008), which are notable for causing lipid peroxidation of organic films and harm to basic proteins and DNA (Li and Osborne 2008).

The likely poisonousness of the combined Hym-AgNPs against HeLa carcinoma cells has been analyzed. The HeLa cells were treated with various fixations (50–200%) of nanoparticles for 48 h and afterward MTT examine was utilized to gauge. As the decrease in cell suitability by ~50% (inhibitory concentration = IC<sub>50</sub>) in correlation with the control was accomplished at a portion of 100  $\mu\text{g}/\text{ml}$  of Hym-AgNPs, while the best number of cells were slaughtered at 200  $\mu\text{g}/\text{ml}$  of Hym-AgNPs. In any case, the cytotoxic impacts might be in part because of direct activity of Ag<sup>+</sup> particles discharged from AgNPs. Singh and Ramarao (2012) detailed that AgNPs were taken-up by macrophages through receptor-intervened phagocytosis and Ag<sup>+</sup> particles were discharged from AgNPs. The free Ag<sup>+</sup> particles thus may meddle with a few cytoplasmic structures and pathways, including mitochondrial capacities actuating pressure pathways and apoptosis. Along these lines, further examinations are expected to light up the AgNP actuated apoptosis and altered form from the viewpoint. In the current examination, to manufacture AgNPs of 50–100 nm in hydrodynamic distance across, which is an extensively littler size for testing cyto-harmfulness. As recently revealed by Sohaebuddin et al.

(2010), nano sized AgNPs are more harmful than bigger and micro sized particles. Also, the AgNPs are synthesized in this examination are very cytotoxic against HeLa cells. At present malignant growth kills around 70,00,000 individuals worldwide on yearly premise. Henceforth, as of late, the quest for the malignant growth therapeutics from normal items expanded step by step. Bioactive mixes in marine life forms have been accounted for against different malignant growth cell lines. Taking everything into account, anticancer movement of silver nanoparticles incorporated by *Hypnea musciformis* appeared impressive cytotoxic impact against human malignant growth cell lines. In this way, the incorporated silver nanoparticles could be considered as a successful anticancer specialist. Be that as it may, an examination in regards to cooperation of microbial orchestrated AgNPs with the disease cell lines should be determined before the broad utilizing clinical applications.

### **5.5 Effect of Green Synthesized Nanoparticles (*Rhp*-AgNPs, *Grc*-AgNPs, *Hym*-AgNPs, *Srt*-AgNPs, *Stm*-AgNPs and *Dtd*-AgNPs) on Soil Microbial Isolates**

Soil quality is characterized as the limit of mud to work, inside normal or oversaw biological system limits, to continue plant and creature efficiency, keep up and upgrade water and air quality, and bolster human wellbeing and home (Karlen et al. 1997). Among the elements impacting soil quality, natural pointers revealed as basically significant (Doran and Zeiss 2000) on the grounds that dirt creatures reasonably impact soil biological system process, particularly the deterioration of soil natural issue and the cycling of supplements (Kennedy and Smith 1995). In this way, security of soil microbial biomass and assorted variety is one of the significant difficulties for economical asset use due to more prominent degrees of microbial biomass and decent variety mean more noteworthy supplement turnover and illness suppressiveness of the dirt (Janvier et al. 2007). The inverse being valid for wiped out soil with low supplement and carbon saves and more prominent degree of contaminants brought about by the nearness of xenobiotics synthetic substances or different variations in the dirt condition. Among the xenobiotics amazing quantities of new nanoparticles designed for modern and natural application or framed by results of human movement, which are as of now finding their way into soils (Maurice and Hochella 2008). While the centralization of most built nanoparticles in the situations despite everything stay obscure, introduction displaying recommend that is soil could be a significant sing of nanoparticles discharged in to the earth and that focus in soil are higher than in water or air (Gottschalle et al. 2009). Built/synthesized nanoparticles can be made of single components like carbon (C) or Silver (Ag) or a blend of components. The expanding section of these nanoparticles unavoidably leads to their amassing in soil, which have raised worries about their aggregation on soil microbial action and decent variety. Right now almost no data is

accessible on how these NPs influence the filth microbial network. Henceforth, the impact of Rhp-AgNPs, Grc-AgNPs, Hym-AgNPs, Srt-AgNPs, Stm-AgNPs and Dtd-AgNPs on the microbial disconnected from garden soil were examined. The detachment of microorganism was completed utilizing sequential weakening strategy. Aliquots of 100  $\mu$ l of various weakening of nursery soil were spread onto plates of supplement agar mode for microorganisms and potato dextrose agar for growths. The plates were brooded at 28 °C for 5 days under oxygen consuming conditions. Created provinces were picked and disengaged dependent on morphological models and the separated microbes were sub-refined as unadulterated culture. Unadulterated refined organisms were identified as *Bacillus spp*, *Bacillus subtilis*, *Staphylococcus epidermidis*, *Serratia spp*, *Pseudomonas spp*, *Pseudomonas fluorescens* and isolated parasites were *Aspergillus disinfects*, *Aspergillus flavus*, *Alternaria alternata*, *Cladosporium spp*. The microscopic organisms detached from the nursery soils are soil N-cycle, nitrifying microorganisms (Mishra and Kumar 2009). To consider the impact of silver nanoparticles on soil organisms to sort of in vitro measure were completed they are calorimetric stock test and agar well dispersion examine.

## 5.6 Calorimetric Chowder Assay

Antimicrobial action of *Rhp* AgNPs, *Grc*-AgNPs, *Hym*-AgNPs, *Srt*-AgNPs, *Stm*-AgNPs and *Dtd*-AgNPs were concentrated against *Bacillus spp.*, *Bacillus subtilis* Gram positive soil nitrifying microbes *Staphylococcus epidermidis* Gram positive denitrifying microscopic organisms, *Serratia spp* is Gram negative soil bio control operator, *Pseudomonas fluorescens*, *Pseudomonas spp* Gram negative, plant development enhancer and biocontrol specialist. *Aspergillus fumigatus*, *Aspergillus flavus*, *Alternaria alternata*, and *Cladosporium spp*, soil borne growths, recycler of carbon and nitrogen from perished living being.

Test of 3 ml of microbial culture were put into test cylinders and 1, 1.5 and 2  $\mu$ l of proper weakening of *Rhp*-AgNPs, *Grc*-AgNPs, *Hym*-AgNPs, *Srt*-AgNPs, *Stm*-AgNPs and *Dtd*-AgNPs were included. Following 24 hours brooding, absorbance perusing at 520 nm frequency for every growth were estimated post hatching at 37 °C for 12 hours. Bacterial cell practicality and least inhibitory focus values were controlled by watching the turbidity and the absorbance perusing of the suspension post brooding. The most minimal centralization of blended nanoparticles with clear suspensions was considered as the esteems. The suspensions of disconnected microbial (*Bacillus spp*, *Bcillus subtilis*, *Serratia spp*, *Pseudomonas fluorescens*, *Pseudomonas spp*, *Staphylococcus epidermidis*, *Aspergillus disinfects*, *Aspergillus flavus*, *Alternaria alternata* and *Cladosporium spp*) inoculums with every extraordinary focus (1  $\mu$ l, 1.5  $\mu$ l, 2  $\mu$ l) of *Rhp*-AgNPs, *Grc*-AgNPs, *Hym*-AgNPs, *Srt*-AgNPs, *Stm*-AgNPs and *Dtd*-AgNPs in stock examination technique were exceptionally overcast that stayed all through the hatching time frame. This watched the visual suspension for deciding the MIC as the turbidity because of bacterial and contagious development.



The calorimetric stock microbial development examination gave MIC esteem is 1  $\mu$ l for all the tried 6 microbes and 4 parasites. The lower MIC values got for microorganisms was *Pseudomonas fluorescens* and *Serratia spp* against *Rhp*-AgNPs, *Grc*-AgNPs, *Dtd*-AgNPs of which *Dtd*-AgNPs indicated most extreme development inhibitory action against *Pseudomonas fluorescens*. The consequences of calorimetric stock microbial development examine of silver nanoparticles orchestrated from ocean growth indicated moderate inhibitory impact against a large portion of the dirt confined parasites which differed in the range. The MIC esteems were seen in *Rhp*-AgNPs and *Grc*-AgNPs. The secluded fungal micro-organisms *Aspergillus fumigatus*, *Aspergillus flavus*, *Alternaria alternata* and *Cladosporium spp* had supplementary reticence (less turbid mixture) on *Rhp*-AgNPs and *Grc*-AgNPs. The results of our revision showed momentous antimicrobial behavior in calorimetric broth assay.

## 5.7 Agar Well Diffusion Assay

The impact of silver nanoparticles blended by eatable ocean growth on the microorganisms disconnected from the nursery soil were concentrated by calorimetric stock test and further affirmed by Agar well dispersion examination. The consequence of the antimicrobial screening trial of *Rhp*-AgNPs, *Grc*-AgNPs, *Hym*-AgNPs, *Srt*-AgNPs, *Stm*-AgNPs and *Dtd*-AgNPs were tried against soil microbial segregates (*Bacillus spp*, *Bcillus subtilis*, *Serratia spp*, *Pseudomonas fluorescens*, *Pseudomonas spp*, *Staphylococcusepidermidis*, *Aspergillus fumigatus*, *Aspergillus flavus*, *Alternaria alternata*, and *Cladosporium spp*) utilizing agar well dispersion procedure. The blended AgNPs were seen as increasingly compelling against tried microbes. *Grc*-AgNPs, *Hym*-AgNPs, *Srt*-AgNPs, *Stm*-AgNPs and *Dtd*-AgNPs were seen as insufficient or demonstrated helpless restraint on bacterial and contagious development. The bigger zone of hindrance was watched for *Rhp*-AgNPs against *Serratia spp* (8 mm) and *Cladosporium spp* (9 mm).

Despite the fact that base hindrance on soil microorganisms by orchestrated silver nano particles have been seen in our examination. The possibility that microorganisms are safe, resilient, and practically repetitive is invasion in biology (Allison and Martiny 2008). Serious extent of metabolic adaptability, physiological resilience to changing ecological conditions (Mayer et al. 2004), high bounties broad dispersal, and potential for quick development rates have likewise lead to the proposal that microbial networks will impervious to change (Fenchel and Finlay 2004). Furthermore, fast developmental adjustment through flat quality exchange could permit touchy microorganisms to adjust to new natural conditions and rapidly return the network to its unique piece (Allison and Martiny 2008). Obviously, these examinations show that filth microbial networks frequently are very strong to bothers. Despite the fact that far reaching comprehension of the associations between metal oxide NPs and microorganisms particularly microbes is still at an early age (Han and Gu 2010), Findings propose that microscopic organisms with a capacity to bear a

poisonous specialist may show up with time (Baath 1992) and the antimicrobial movement of incorporated NPs might be decreased by bacterial self security component; for example, *Bacillus subtilis* (Gram positive) reacted to nC60 by modifying layer lipid arrangement, stage progress temperature, and film smoothness (Fang et al. 2007). Earlier, Thill et al. (2006) contemplated the effect of water scattering of CeO<sub>2</sub>-NPs (7 nm) on Gram negative bacterium *E. coli* and discovered emphatically charged CeO<sub>2</sub> at nonpartisan pH showed a solid electrostatic fascination towards bacterial external layers and Ce (1 V) was diminished to Ce (111) at the outside of the microorganisms. Microscopic organisms additionally have other defensive reactions (Wu et al. 2010), they demonstrated that bacterial cells have extra cell protein to kill limited quantities of poisonous particles to microorganisms and such defensive components might turn out to be less compelling. Sudheer Khan et al. (2011) found that microscopic organisms emitted exopolysaccharides that topped AgNPs in this manner decreasing its harmfulness to *E.coli*, *S. aerues* and *Micrococcus luteus* contrasted with the uncapped ones. The AgNP-harmfulness to nitrification microscopic organisms has been accounted for to be exceptionally reliant on their size, where AgNPs with under 5 nm distance across were accounted for to fundamentally restrain the nitrification microbes (Cha and Hu 2008). Our outcome indicated that the normal molecule size in 20 to 100 nm. AgNPs communications with microorganisms have been seen as reliant on the size and states of the NPs. AgNPs have round (7 and 29 nm) and Pseudo spherical shape (89 nm) with a limited size appropriation. Among these, Martinez-Castanon et al. (2008) found that the 7 nm AgNPs introduced best movement against *E. coli* and *S. aureus*. As a result of their size, 7 nm AgNPs can without much of a stretch arrive at the atomic contact of microscopic organisms and they present the best surface region; in this manner the contact with microbes is the best (Lok et al. 2006). Essentially, the littler size they are, the more noteworthy their surface zone to volume proportion and higher their microbial reaching proficiency (Wong et al. 2010).

## 5.8 Effect of Silver Nanoparticles on Soil Parameters and Earthworms (Morphology and Histology)

Nanoparticles are utilized in science and medication in a wide assortment of ways, including direct application to patients (Salata 2004). Toward the finish of their item life, NPs are probably going to wind up in the earth, especially in soil and water bodies, and consequently influence life forms in those media. NPs have extraordinary diffusivity and along these lines may cause progressively visit contacts with the surfaces of permeable media contrasted with bigger measured particles (Wiesner et al. 2006). Some metal NPs bear novel and exceptional organic properties that permit them to connect explicitly with chosen proteins and restrain their exercises (Bhattacharya and Mukherjee 2008). NPs can likewise infiltrate numerous sorts of cells and tissues, and travel through the body framework causing tissue harm. Silver

(Ag), a respectable metal, has been utilized over numerous years as unadulterated silver (Ag), silver nitrate ( $\text{AgNO}_3$ ), and silver sulfadiazine (Ag SD) for the treatment of consumes, wounds, and a few bacterial contaminations. In any case, because of the rise of progressively powerful anti-infection agents, the utilization of these Ag mixes has declined especially. As of late, nanotechnology has increased huge driving force because of its capacity to deliver metals at nano dimensions, which radically changes the concoction, physical, and optical properties of metals (Bhattacharya and Mukherjee 2008; Srivastava et al. 2021) and the current utilization of such items is expanding. In this manner, concentrates on the eco-toxicology of Ag NPs-one of the most utilized NPs-have gotten increasingly significant.

Night crawlers are key life forms in earthly biological systems. *Lumbricus rubellus* is the most well-known worm species found in farming biological systems (Perez-Losada et al. 2009); consequently, this species is a fascinating contender for use as an animal model to screen soil contamination. So analyzes were completed on *Lumbricus rubellus* (night crawlers), are gathered from Aniyaparanallur, Srivaikundam, Thoothukudi soil. Night crawler of grown-up, weighing around 200–300 mg and having very much evolved clitellum were utilized for all tests and they were developed in earthen pots containing local soil to keep up physical and synthetic boundaries of the dirt. A set with no application were kept up as control. The reference control likewise kept with the utilization of SWC (*Gracilaria corticata* and *Hypnea musciformis*). Another set kept with the utilization of *Grc*-AgNPs and *Hym*-AgNPs are considered as treatment.

Soil tests were drawn when *Gracilaria corticata*, *Hypnea musciformis*, *Grc*-AgNPs and *Hym*-AgNPs application to get to physical (dampness substance and mass thickness and synthetic (pH and natural issue) boundaries of the muck. Information shows that filth dampness content was diminished in the *Grc*-AgNPs regarded soil as days advanced. There is no adjustment in soil dampness with control and soil getting SWC (*Grc* and *Hym*). A pattern of reduction of mass thickness was seen with the expansion of SWC and *Grc*-AgNPs and *Hym*-AgNPs were recorded and were expanded with long periods of medicines.

The materials properties of soil, for example, pH and natural issue were contemplated and organized, natural issue content is for the most part viewed as one of the key pointers of soil quality (Schjonning et al. 2009). Numerous highlights of good soil structure, for example, security, friability and dampness maintenance, might be influenced by soil natural issue. The dirt natural issue expanded continuously in the control and reference control as days continued. However, utilization of SWC expanded the natural issue in all the dirt examples and the addition was fast with expanding development period. On twentieth day, use of *Hypnea musciformis* upgraded the natural issue more effectively than *Gracilaria corticata*. In any case, the degree of soil natural issue was appeared to be decline in the NPs rewarded soil. Diminished dampness content, Bulk thickness and natural issue actuated by silver nanoparticles decreased via growth in the current examination is reliable with prior reports (Cornelis et al. 2012; Benoit et al. 2013; wang et al. 2013).

Soil with fine surface was accounted for to show higher surface territories which additionally encourage Ag-sorption (Jacobson et al. 2005). Ag sorption and

versatility are likewise constrained by soil natural matter (Jones and Peterson 1986). Soils with high natural issue fixations absorb Ag more firmly than to mineral soils. To acquire understanding into the impact of AgNPs in the earthbound condition, *Lumbricus rubellus* worm sort, presented to Ag-NPs, and morphology and histology were seen on each twentieth and 40th days. One lot of reference control were kept with use of SWC.

The introduction of *Grc*-AgNPs and *Hym*-AgNPs had no impact on worm endurance over the multi day time frame. There was 100% endurance at both the nanoparticles introductions. Our outcomes are in concurrence with the discoveries of Roh J-Y et al. 2009. Be that as it may, following 20 days of presentation of AgNPs the earthy colored pigmentation of worm's skin step by step changed into dim shading. The pigmentation is bit by bit diminished with expanded period in all the NPs rewarded night crawler.

Impact of silver nanoparticles introduction on the life structures of the night crawler likewise examined and the transverse segment of fragments from the clitellum area of the control, SWC rewarded and *Grc*-Ag nanoparticles uncovered worms were taken fingernail skin and epidermis of the all the tried night crawler were unblemished. The roundabout and longitudinal muscles are unaffected. In any case, shrinkage of digestive tract divider is seen in the *Grc*-AgNPs uncovered worms.

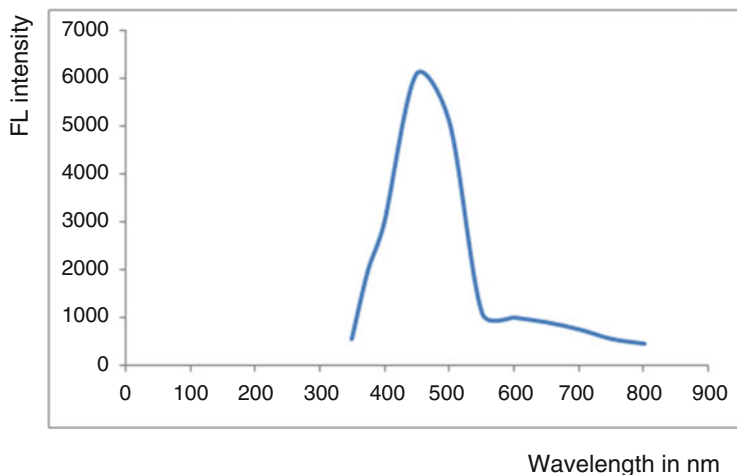
The histology concentrates on worm gave the end that the green incorporated nanoparticles indicated high harm in the intestinal divider. No different variations from the norm were seen in charge and reference control classification. The AgNPs saw as having negative impact on the night crawler.

## 5.9 Fluorescence—FL

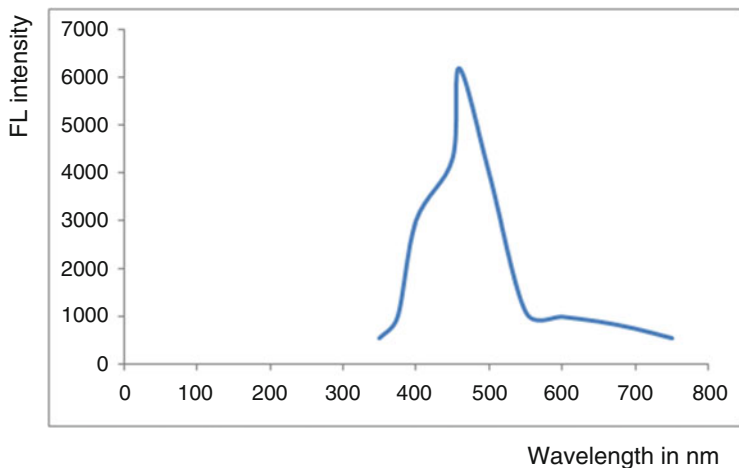
Atoms have different states alluded to as vitality levels. Fluorescence spectroscopy is principally worried about electronic and vibrational states. For the most part, the species being analyzed has a ground electronic express (a low vitality condition) of intrigue, and an energized electronic condition of higher vitality. Inside every one of these electronic states there are different vibrational states. In fluorescence, the species is first energized, by engrossing a photon, from its ground electronic state to one of the different vibrational states in the energized electronic state. Impacts with different particles cause the energized atom to lose vibrational vitality until it arrives at the most reduced vibrational state from the energized electronic state. The particle at that point drops down to one of the different vibrational degrees of the ground electronic state once more, radiating a photon in the process. As atoms may drop down into any of a few vibrational levels in the ground express, the produced photons will have various energies, and along these lines frequencies. Consequently, by breaking down the various frequencies of light discharged in fluorescent spectroscopy, alongside their relative forces, the structure of the diverse vibrational levels can be resolved. An emanation map is estimated by recording the outflow spectra

**Table 5.1** FL data of six types of silver NPs

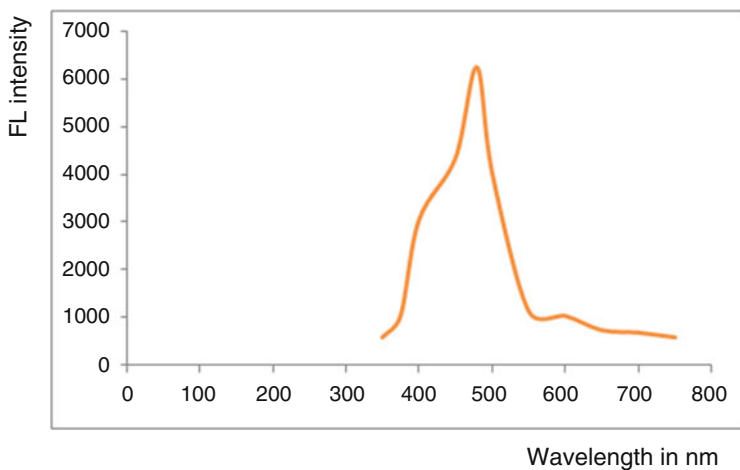
Nano samples	FL emission (nm)	Band gap value (eV)	Colour
(a) <i>Rhp</i> -AgNPs	449	2.7616	Violet
(b) <i>Grc</i> -AgNPs	459	2.7015	Blue
(c) <i>Hym</i> -AgNPs	479	2.5887	Blue
(d) <i>Srt</i> -AgNPs,	490	2.5306	Bluish green
(e) <i>Stm</i> -AgNPs	493	2.5152	Bluish green
(f) <i>Dtd</i> -AgNPs	497	2.4949	Bluish green

**Fig. 5.4** FL spectra of the synthesised silver nanoparticles from the aqueous extracts of seaweeds (a) *Rhp*-AgNPs at 449 nm

coming about because of a scope of excitation frequencies and joining them all together. This is a three dimensional surface informational index: outflow power as an element of excitation and discharge frequencies, and is normally portrayed as a form map. The FL behaviour of the six samples of silver nano particles have the FL emission wavelengths of 449, 459, 479, 490, 493 and 497 nm respectively for (a) *Rhp*-AgNPs (b) *Grc*-AgNPs, (c) *Hym*-AgNPs (d) *Srt*-AgNPs, (e) *Stm*-AgNPs (f) *Dtd*-AgNPs and they have the band gap of 2.7616, 2.7015, 2.5887, 2.5306, 2.5152 and 2.4949 eV and shows the violet FL for 449 nm and bluish FL for 459, 479 nm and 490, 493 and 497 shows the bluish green emission which factors the emission for the excitation values and also shows the nano sample variance of transmittance and FL value for the colour change observed in different silver NPs and is mentioned in Table 5.1 and Figs. 5.4, 5.5, 5.6, 5.7, 5.8, 5.9.



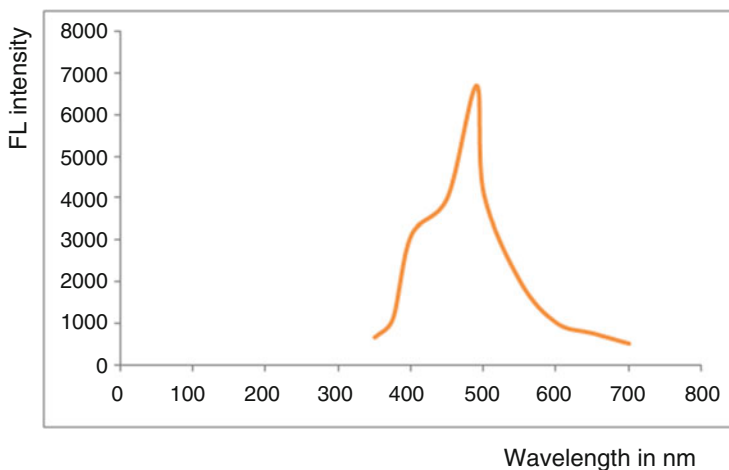
**Fig. 5.5** FL spectra of the synthesised silver nanoparticles from the aqueous extracts of seaweeds (b) *Grc*-AgNPs at 459 nm



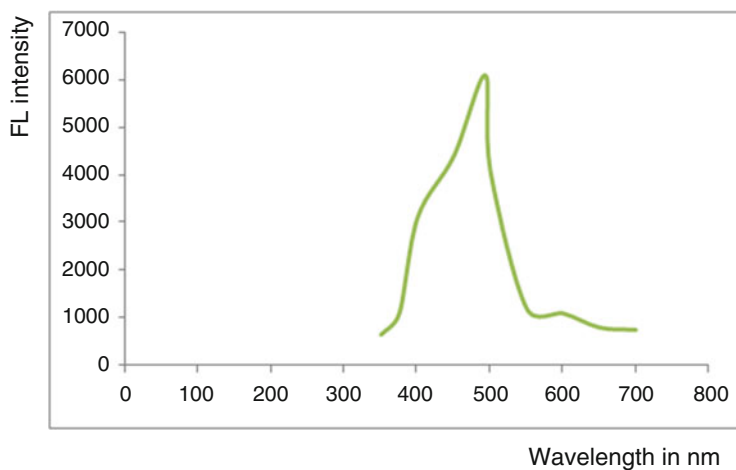
**Fig. 5.6** FL spectra of the synthesised silver nanoparticles from the aqueous extract of seaweeds (c) *Hym*-AgNPs at 479 nm

## 5.10 Conclusion

Seaweed extracts have been used for the synthesise of silver nanoparticles (Ag-NPs) as they offer many completion in versatile fields. The use of biological materials in nanoparticles synthesis is preferred as they are eco-friendly and compatible for pharmaceutical and other biomedical applications, here antioxidant and anti-cancerous effect is discussed. Silver metal has toxicity against a wide range of microorganisms especially silver nanoparticles which has promising antimicrobial

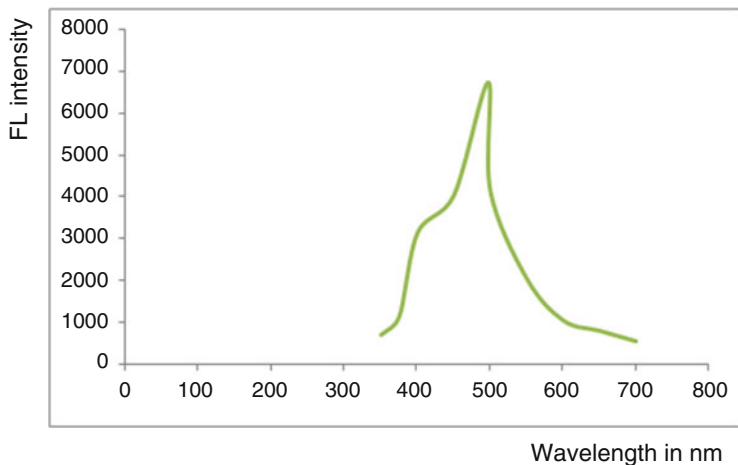


**Fig. 5.7** FL spectra of the synthesised silver nanoparticles from the aqueous extract of seaweeds (d) *Srt*-AgNPs at 490 nm



**Fig. 5.8** FL spectra of the synthesised silver nanoparticles from the aqueous extracts of seaweeds (e) *Stm*-AgNPs at 493 nm

properties. Silver nanoparticles are effective as anti-inflammatory, anti-angiogenesis, antiviral and anti-platelet activity against cancer cells. The Ag-NPs synthesized were from the aqueous extracts of seaweeds and were characterized by using spectral analysis. The effect of the synthesized silver nanoparticles on the isolated soil microbes were assessed by different assays such as Calorimetric chowder assay and Agar diffusion assay. The effect of synthesized Ag-NPs on earthworm was also studied by examining its morphology and histology. The toxicity of the combined seaweed-AgNPs against HeLa carcinoma cells has been analyzed and FL data



**Fig. 5.9** FL spectra of the synthesised silver nanoparticles from the aqueous extracts of seaweeds (f) *Dtd*-AgNPs at 497 nm

analyze the band gap and color of FL for each specimen and shows that the synthesized Ag-NPs are free from flaws.

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