

Lema, Y & Asnake, L. *Sci. Technol. Arts Res. J., Oct.-Dec. 2023, 12(4), 129-146* DOI:<https://doi.org/10.20372/star.v12i4.10> ISSN: 2226-7522 (Print) and 2305-3372 (Online) Science, Technology and Arts Research Journal

Sci. Technol. Arts Res. J., Oct. – Dec. 2023, 12(4), 129-146

Journal Homepage: https://journals.wgu.edu.et

Review Article

A Review on Green Synthesis, Characterization and Application of Metal Nanoparticles

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Abstract Article Information *Atoms or molecules having unusual physical properties and a size between one and one hundred nanometers are called nanoparticles (NPs). Their origins, sizes, and structural arrangements allow them to be grouped into different categories. Two approaches are utilized in the synthesis of NPs: the top-down strategy and the bottom-up procedure. The top-down size reduction method is used to make NPs by dividing the bulk material into tiny particles. Developing NPs from smaller building components is done via a bottom-up technique. To synthesize, the bottomup method incorporates both chemical and biological procedures. The green synthesis method is among the most well-liked bottom-up approaches to NP production. Biomaterials derived from various sources are utilized, such as bacterial, fungal, algal, and plant extracts. Investigation of NPs can be carried out using state-of-the-art nano-characterization techniques such as UV-vis, AFM, TEM, SEM, XRD, and FT-IR after synthesis. Soil health and productivity were preserved for centuries to come because to nanoparticles' uses in biomedicine, antimicrobial agents, cancer treatments, catalysis, and other areas. Nanoparticles, bottom-up*

approach, top-down approach, green synthesis, characterization, Applications *Corresponding Author:

Article History:

Received : 02-10-2023 Revised : 30-11-2023 Accepted : 27-12-2023

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Keywords:

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INTRODUCTION

According to Senapati (2005), nanotechnology is the practice of using scientific methods to control the molecular properties of materials. The size range of solid atomic or molecular particles known as nanoparticles (NPs) is 1– 100 nm. They differ from bulk molecules in a number of important physical respects, depending on their size and shape. The chemical industry, electronics, photoelectrochemical applications, optoelectronic

devices, energy research, optics, mechanics, medicine, and space industries are among the many areas where it is gaining prominence (Hoffman et al., 1992). It has the potential to produce nanoscale (less than 10 nm) geranium quantum dots under controlled conditions. To be more precise, what allows for the creation of quantum dot lasers through the simple manipulation of nanoparticle size (Matson & Wilson, 2010). The unique mechanical, biological, steric, thermal, electrical, absorption, and melting points of

nanoparticles in comparison to bulk materials of the same chemical make-up make them fascinating. Their high surface-to-volume ratio and diminutive size were noted by Zharov et al. (2005).

 Therefore, novel materials with novel uses can be created by controlling size and shape at the nanoscale. In addition, these nanoparticles have several uses, such as antibacterial properties, medicine delivery, medical imaging, computer transistors, filters, and chemical sensors (Pissuwan, 2006). When it comes to biomedical applications and the emerging multidisciplinary discipline of nanobiotechnology, nanoparticles crafted from gold and silver are by far the most popular choices. A number of nanoparticles are used in the military, animal husbandry, packaging, accessories, health, and medical fields because of their antibacterial properties (Duran et al., 2007). The ever-increasing demand for nanoparticles has prompted a wide range of approaches to their synthesis and optimization (Vijayan et al., 2016). Unfortunately, these traditional methods are not eco-friendly since they include expensive and harmful chemicals. Due to its stability, affordability, clinical adaptability, biocompatibility, and environmental friendliness, biogenic production and microorganisms are currently attracting a lot of attention from researchers (Ahmed et al., 2016). A wide range of plants and microbes, such as actinomycetes, algae, bacteria, fungus, viruses, and yeast, are involved in biosynthesis, an eco-friendly green chemistry process (Kumar et al., 2011). Biological entities can be utilized as biological factories to produce nanoparticles of diverse sizes, shapes, compositions, and physicochemical qualities in a way that is

Lema, Y & Asnake, L. *Sci. Technol. Arts Res. J., Oct.-Dec. 2023, 12(4), 129-146* safe, non-toxic, and ecologically friendly (Khan et al., 2013). In contrast to microorganisms, plants do not necessitate any special, complicated, or multi-stage procedures for isolation, culture preparation, or culture maintenance, which is a major benefit of the plant approach. A brief overview of the procedures used to synthesize and characterize nanoparticles, as well as their potential applications, is the goal of this work.

SYNTHESIS METHODS OF METAL NANOPARTICLES

Figure 1 shows the two main ways to nanoparticle synthesis: the top-down method and the bottom-up method. Ahmed et al. (2016) states that in order to create nanoparticles, the top-down method involves size-reduction of the bulk material. This procedure can be carried out utilizing a range of physical and chemical methods, including as photoreduction, chemical etching sputtering, thermal evaporation, mechanical processes (such milling and grinding), and lithography. Nevertheless, surface structure imperfection is the primary downside of the top-down strategy (Bukka et al., 2019). A process known as bottom-up synthesis, the self-assembly approach creates nanoparticles from atoms, molecules, and even smaller particles. The green synthesis of nanoparticles is one of the processes covered by the bottomup method. Shah et al. (2015) and Yahya et al. (2019) found that this technique does not involve harsh, dangerous, or expensive chemicals, making it ecologically friendly. Chemical synthesis is the gold standard for creating metallic nanoparticles, but it has limited uses because it requires expensive and

potentially harmful compounds, such as those for reducing and agent stabilizing. In biological contexts, these nanoparticles could potentially cause harm as well. Since this is the case, there is a rising need to find safe, cost-effective ways to manufacture

Lema, Y & Asnake, L. *Sci. Technol. Arts Res. J., Oct.-Dec. 2023, 12(4), 129-146* nanoparticles. Consequently, the purpose of this study is to analyze a large body of literature concerning the use of microorganisms and plant extracts in the production of environmentally friendly nanoparticles (Table 1).

Figure 1 *Numerous methods for synthesising nanoparticles* (Yahya, et al., 2019)

Table 1

Categories	Method	Nanoparticles
Bottom-up	Sol-gel Carbon	metal and metal oxide based
	Spinning	Organic polymers
	Chemical Vapour Deposition	Carbon and metal based
	(CVD)	
	Pyrolysis	Carbon and metal oxide based
	Biosynthesis	Organic polymers and metal
		based
Top-down	Mechanical milling	Metal oxide and polymer
		based
	Nanolithography	Metal based
	Laser ablation	Carbon based and metal oxide
		based
	Sputtering	Metal based
	Thermal decomposition	Carbon and metal oxide based

Groups of the nanoparticles created using the different techniques.

Green Synthesis of Nanoparticles

 Microorganisms in Synthesis of Nanoparticles

Many microbes, such as bacteria, fungi, algae, and yeast, have the ability to synthesize NPs. Bacteria have enzymes that help them collect and break down toxic metal ions into smaller, more manageable particles called

nanoparticles. In most cases, NPs can be produced by microbes either inside or outside of cells (Ravichandran et al., 2016). One way to closely observe the biosynthetic process is to watch how the color of the culture media changes (Singh et al., 2016). Media have an effect on NP yield and response.

Figure 2 *Mechanism of metal nanoparticle creation from microorganisms represented mechanistically.*

A variety of post-treatment processes can be employed to produce the final nanoparticles (NPs), including centrifugation, washing, drying, and collection. Centrifugation is used to collect the bacterial biomass, which is then dissolved in the metal salt solution and incubated for intracellular synthesis. After the incubation period, the biomass can be extracted using centrifugation, washing, and mechanical disruption or ultrasonication, among other methods (Singh et al., 2016).

Lema, Y & Asnake, L. *Sci. Technol. Arts Res. J., Oct.-Dec. 2023, 12(4), 129-146* Even though it's easier to access extracellular synthesis than intracellular synthesis, residual fermentation does take place (Figure 2).

Fungi in Nanoparticle Synthesis

Fungi outperform other biosynthetic processes in NP production due to their tolerance and bioaccumulation capacity (Zhao et al., 2018). Most studies use extracellular methods because NPs outside of cells tend to be smaller than those within (Table 2).

Table 2

Bacteria in Nanoparticle Synthesis

Because of their ease of use, environmental friendliness, and capacity for large-scale production, bacteria were the first microorganisms to be employed in the early development of biosynthesis (Table 3). It is the most widely used technique for biosynthesis. Numerous bacteria, including *Streptomyces bikiniensis, Bacillus subtilis,*

In order to create various kinds of nanoparticles (NPs), which are produced when microorganisms react with metal ions and chemically reduce them to biologically stable NPs, researchers have looked at Veillonella atypica, Cupriavidus metallic durans, Aspergillus terreus, Rhodo pseudomonas capsulate, and Escherichia coli (Saravanan et al., 2018).

Bacterial biosynthesis of nanoparticles (Hulkoti & Taranath, 2014)

Yeasts in Nanoparticle Synthesis

A large number of researchers have investigated yeasts as a potential biogenic source for nanoparticles. Yeast has an innate ability to gather and retain enormous clusters of harmful metal particles from its environment. As shown in Table 4, yeast cells adapt to environments with metal toxicity by

employing a number of detoxifying mechanisms, such as bio-precipitation, chelation, and intracellular sequestration. Sarkar and Acharya (2018) and Gahlawat et al. (2017) are only two examples of how researchers have used this property of yeast cells.

Table 4

Yeast-based biosynthesis of nanoparticles (Hulkoti & Taranath 2014).

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Algae in Nanoparticle Synthesis

Some research have shown that, like yeast, algae may be utilized to make metal nanoparticles in a controlled environment (Table 5). Silver nanoparticles ranging in size from 9.8 to 5.7 nm were synthesized by Da Silva Ferreira et al. (2017) using chlorella

vulgaris. An environmentally friendly alternative to conventional antimicrobials with promising biological potential. Furthermore, Momeni and Nabipour (2015) looked into the production of silver chloride nanoparticles by means of the aquatic extract of the marine alga Sargassum plagiophyllum.

Table 5

Different algae species engaged in the creation of nanoparticles (Anu et al., 2020)

Plants' Extracts in Metal Nanoparticles Synthesis

Recent studies on the biosynthesis of nanometals from plant extracts have ushered in a new era of safe and efficient nanoparticle production technologies. Metal nanoparticle production from plant leaf extracts and its possible applications have been the subject of multiple investigations. Research on the bioreduction of gold and silver ions by Azadirachta indica leaf broth and Pelargonium graveolens was conducted by Chandran et al. (2006). Additionally, Shankar et al. (2004) investigated how lemongrass (Cymbopogon flexuosus) extracts generated triangle nanoprisms of gold. Rapid bioreduction, assembly, and room-temperature sintering of "liquid-like" spherical gold nanoparticles appeared to be the sequence of events that culminated in the formation of the nanotriangles. According to Singh et al. (2020), a process called plant-assisted synthesis The

efficiency of plant-assisted NP synthesis outshines that of microbial synthesis, given a constant yield. During the formation of

Lema, Y & Asnake, L. *Sci. Technol. Arts Res. J., Oct.-Dec. 2023, 12(4), 129-146* biogenic NPs, many plant metabolites and biochemicals, like polyphenoyls, can serve as agents that reduce and stabilize (Table 6).

Table 6

Important examples of nanoparticle biosynthesis using plants (Singh et al., 2020)

Green Synthesis Procedures of Some Selected Metal Nanoparticles

 Green synthesis of Copper Nanoparticles (CuNPs)

According to Amer and Awwad (2021), CuNPs were synthesized using extracts from citrus fruits. After being rinsed with distilled water, the fresh citrus lemon fruit samples were chopped into small pieces and placed in a 250 ml glass beaker with 100 ml of deionized water. The mixture was then heated to 80 oC for ten minutes. The next step was to filter the mixture through Whatman No. 41 filter paper to produce the citrus limon fruit aqueous extract. The experimental procedure involved storing the clear filtrate in a refrigerator-safe glass flask with a cork (Figure 3). A 100 ml aqueous citrus limon fruit extract and 4 g of copper sulphate pentahydrate were swirled magnetically for four hours at room temperature (27 oC). The transformation of copper sulphate pentahydrate's blue color to brown after ten minutes indicated the formation of CuNPs due to the reduction of copper ions from Cu(II) ions to Cu metal. The next step was to spin the samples at 3000 rpm for 10 minutes to separate the liquid, which was then allowed to cool to room temperature. The resulting copper nanoparticles were oven-dried for four hours at temperatures ranging from 80 to 90 degrees Celsius.

Figure 3 *Schematic presentation of CuNPs synthesis and characterization using Citrus lemon fruit extract.*

Preparation of Gold Nanoparticles (AuNPs)

Here is the process for making AuNPs using fresh Mentha Longifolia leaves (Li et al., 2021). A sample of Mentha Longifolia leaf weighing 2.0 g was heated in 100 mL of deionized water to 80°C for 30 minutes. The colored concoction was filtered using Whatman filter paper No. 1 after cooling in order to produce the watery extract. It was stored in the refrigerator at 4˚C until later use. Pour 10 milliliters of Mentha Longifolia leaf aqueous extract dropwise into 50 milliliters of a thoroughly mixed 0.001 M HAuCl4 aqueous solution, stirring constantly at room temperature. This is the conventional method for producing Au NPs. After 20 minutes, the mixture's color changed from pale yellow to wine red, which was a little indication that the Au NPs had been successfully synthesized.

The crystal top was removed by decanting after 20 minutes of centrifugation at 4000 rpm in the solution containing the nanoparticles. The residue that remained after being rinsed with deionized water was dried by baking it at 50 degrees Celsius.

Preparation of Silver Nanoparticles (AgNPs)

Ajitha et al. (2015) produced AgNPs using freshly harvested Latana camara leaves. Hence, new L. camara leaves were collected, rinsed under running water, and subsequently surface washed with Milli-Q water to eliminate any impurities. The dirt-free leaves were let to dry at room temperature for ten days under a shade tent to remove any residual moisture. The powdered dry leaves were ground in a sterile electric blender and then

sealed in a bottle, out of the reach of children and pets, until they were needed again. 10 grams of leaf powder and 100 milliliters of Milli-Q water were thoroughly combined, then heated to 60 degrees Celsius for 10 minutes. After cooling, the mixture was filtered through Whatman No. 1 filter paper to produce LE. Shortly after filtering, the extract was taken out and used in further research. To

Lema, Y & Asnake, L. *Sci. Technol. Arts Res. J., Oct.-Dec. 2023, 12(4), 129-146* synthesize nanoparticles, an Erlenmeyer flask was made to accommodate 100 ml of a waterbased solution of 0.001 M silver nitrate. Five, ten, fifteen, and twenty milliliters of L. camara leaf broth were added to the flask separately while they were at room temperature. As a result, A1, A2, A3, and A4 colloids were synthesized. Here is a visual representation of the schematic synthesis process: Figure 4.

Figure 4 *Schematic procedure of AgNP synthesis in green condition.*

The reaction flasks were kept covered with aluminum foil and stirred continuously for 10 minutes. Within five minutes, the solution had transformed into a brownish yellow hue, signifying the formation of AgNPs. The fact that no more color change was seen further supports the idea that the silver ions had undergone rapid bio-reduction. The resultant AgNPs were purified by redispersing the pellet in Milli-Q water twice following centrifugation at 15,000 rpm for 10 minutes to remove water-soluble biomolecules such proteins and secondary metabolites. The next step was to transfer the AgNPs to a sterile vial for future study.

Preparation of Zinc oxide nanoparticles ZnO NPs

In 2015, Elumalai et al. reported synthesizing zinc oxide nanoparticles using extracts from Moringa oleifera leaves. We used tap water and distilled water to wash the collected M. oleifera, removing any unwanted pollutants including dust, dirt, and other materials. Twenty grams of the dried leaf sample were taken out for synthesis after it had been left to dry at room temperature (32 °C) . The leaves, which weighed 20g, were simmered in 100 mL of double-distilled water at 60˚ C for 20 minutes. During the boiling process, a solution

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of room temperature, with a pale yellow hue, was created. After that, the yellowish extract was filtered through Whatman No. 1 filter paper and put in the fridge. Furthermore, 10 mL of M. oleifera leaf aqueous extract was drawn from the chilled stock solution and, using a magnetic stirrer, heated to temperatures ranging from 60 to 80 degrees

Lema, Y & Asnake, L. *Sci. Technol. Arts Res. J., Oct.-Dec. 2023, 12(4), 129-146* Celsius. The solution was supplemented with two grams of zinc nitrate hexahydrate (Zn (NO3)2.6H2O) once it had reached sixty degrees Celsius. After that, the ingredients were heated until they became a thick, yellow paste. Proceeding to a ceramic crucible cup, it was subjected to two hours of cooking in a furnace set at 400 $^{\circ}$ C (Figure 5).

Figure 5 *The flowchart used for the preparation of ZnO NPs.*

Characterization Methods of Nanoparticles

Several methods are employed to ascertain the dimensions, form, structural make-up, and surface area of nanoparticles. After production, the nanoparticles can be studied using advanced nano-characterization techniques. A few examples of popular microscopy and spectroscopy methods are scanning electron microscopy (SEM), atomic force microscopy (AFM), powder X-ray

diffraction (XRD), and Fourier transform infrared spectroscopy (FT-IR). Several microscopical techniques, such as AFM, SEM, and TEM, provide direct data gathering from images of nanoparticles. The size and morphological features of nanoparticles have been extensively measured using SEM and TEM, in particular (Shah et al., 2015).

Table 7

Methods employed in the characterisation of nanoparticles

Techniques	Characterization of NPs	
UV-vis	Absorbance of the sample	
TEM and SEM	Size and shape	
XRD	Crystalline structure	
AFM	Size distribution	
FT-IR	Phytochemical investigation	

According to Pinern et al. (2013), ultraviolet and visible (UV-Vis) absorption spectroscopy is a way to quantify the light's attenuation either before or after it passes through the material. Electron microscopy (EM) studies often deduce the structural properties of green nanoparticles, including surface morphology, topography, direct visualization, and nanoparticle measurement. Surface electron microscopy (SEM) images reveal a threedimensional view of the particles, in contrast to transmission electron microscopy (TEM) images, which are two-dimensional. Investigating particles by transmission electron microscopy (TEM) reveals their crystallinity and lattice structure, revealing their internal composition and enabling higher magnification and resolution. Images captured by AFM can provide three-dimensional data regarding the nanoparticles' size, shape, and distribution. Infrared spectroscopy (FTIR) provides information about the chemical

Lema, Y & Asnake, L. *Sci. Technol. Arts Res. J., Oct.-Dec. 2023, 12(4), 129-146* characteristics of nanoparticles based on the vibrational fingerprints of specific functional groups (Jayaseelan et al., 2013). According to Husseiny et al. (2007), the crystalline nature of the nanoparticles was ascertained using Xray diffraction (XRD) analysis.

APPLICATION OF NANOPARTICLES

Drug delivery, bioimaging, cancer treatment, medical diagnostics, and sensor development are just a few of the many applications of biologically produced metallic nanoparticles in the biomedical field. These unique properties include not just biocompatibility, stability, and manipulability, but also insulating, optical, antibacterial, antioxidant, and anti-metastasis capabilities. These days, metallic nanoparticles are all the rage in the industrial sector thanks to their catalytic activity. Biologically generated metallic nanoparticles have several potential uses, as shown in Figure 6 (Schrofel et al., 2014).

Figure 6 *Application areas of metallic nanoparticles synthesized by biological methods.*

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Nanoparticles in Catalysis

Due to their exceptional properties, which are a result of their intrinsically large surface-tovolume ratios, modern transition metal nanoparticles (M-NPs) with a restricted size distribution have garnered considerable interest from researchers and businesses alike. According to Kidwai (2010), transition M-NPs are known to possess several desirable catalytic properties.

 While homogeneous catalysis is highly effective and selective, it does come with some downsides, such as a lack of heat stability and difficulty in extracting the catalyst from the reaction medium. Heterogeneous catalysis offers simplicity in catalyst material recovery and the ability to use high temperatures compared to homogeneous catalysis, but it has poor selectivity and necessitates an in-depth understanding of the mechanisms involved, which are critical for optimizing parameter values (Astruc, 2008). However, M-NPs play a crucial role in catalysis by replicating nanoscopic-scale metal surface activation and catalysis, which enhances the selectivity and efficiency of heterogeneous catalysis.

Sensor Application of Nanoparticles

Green technology has hindered the potential use of AuNPs as a sensor candidate. Beyond its more conventional applications in measuring things like pressure, temperature, and flow, they will find widespread use in both domestic and commercial settings. These encompass a wide range of commonplace

Lema, Y & Asnake, L. *Sci. Technol. Arts Res. J., Oct.-Dec. 2023, 12(4), 129-146* activities as well as vehicles, planes, spacecraft, robots, and machinery. Adamu and Osama (2019) provide the most up-to-date information regarding the biosynthesis and detection capabilities of AuNPs.

Nanoparticles' Antioxidant Capacity

Chemists produce free radicals during oxidation, which may trigger a cascade of events that damage cells in the body. To keep intricate overlapping systems running smoothly and to control the oxidative state in plants and animals, antioxidants like ascorbic acid and thiols interrupt these chain reactions. The damage caused by oxidation can be partially reversed and protected by this endogenous production (Chen et al., 2016). As a result, a number of scientists are interested in studying the antioxidant properties of NPs synthesized from bioagent materials.

Antimicrobial Activity of Nanoparticles

Biosynthesized nanoparticles were initially studied for their antibacterial capabilities in 2007 by Vigneshwaran et al. Their antifungal, anticorrosive, and antibacterial actions are attributed to their larger specific surface area, smaller particles, higher surface reactivity, abrasive surface roughness, and surface imperfections. Three separate pathways may account for metal nanoparticles' antibacterial effects: first, oxidative stress; second, damage to intracellular microbial components subsequent to cell wall penetration; and third, damage to cell walls and membranes. Figure 7 below shows the processes that cause metal nanoparticles to kill bacteria.

Figure 7 *Prospective mechanisms for antimicrobial activity for metallic NPs.*

Drug Delivery and Anticancer Activity of Nanoparticles

How well and efficiently a medicine works depends on how well and how specifically it dissolves its target. Additional doses of a treatment must be associated with negative pharmacological reactions in order to treat illnesses in human cells. Modern medicine has moved away from radiation, chemotherapy, and surgery in favor of more creative methods of treatment. Gold and silver NPs are among the novel NPs that have emerged as possible tools for the regulated and sustained distribution of different therapeutic compounds to specific locations (Bhat et al., 2017). Because of its possible usage in cancer therapies and other medical issues, gold nanoparticles have piqued the curiosity of researchers. For the successful development of AuNPs, fruit extract from Genipa Americana L. was utilized. At room temperature, the

AuNPs remain stable for more than six months and do not contain any harmful chemicals. According to Rajan et al. (2016), it demonstrated promising results when tested against HeLa, cervix A-549, and lung cancer cells.

CONCLUSIONS

Nanoparticles and their eco-friendly manufacturing, characterization, and use were the focus of this review. Two primary approaches exist for synthesizing nanoparticles: the top-down methodology and the bottom-up approach. The top-down approach to creating nanoparticles involves shrinking the substance to minuscule particles, which then separates them from the larger material. Physical and chemical methods are both capable of achieving this goal. However, the uneven surface structure is the primary drawback of the top-down technique. The

other method, called the bottom-up strategy, constructs nanoparticles from less substantial components. Methods from the bottom up approach encompass chemical and biological synthesis. Nanoparticle production by biosynthesis has been the subject of extensive study. The biological synthesis of NP requires inexpensive technologies, solvents that are safe for the environment, renewable resources that are readily available, compounds that are not poisonous, and clean. So, our review scoured the literature for information on how to make nanoparticles using microbes and plant extracts in an eco-friendly way. Compared to other forms of life, the metal nanoparticles produced by plants are more stable, as stated in the review. When it comes to extracts from plants, plants can decrease metal ions at a faster rate than bacteria and fungus. Nanoparticles can be studied by employing advanced nano-characterization techniques. Common techniques in spectroscopy and microscopy include: FT-IR, XRD, UV-vis, AFM, TEM, SEM, and SEM. Biologically generated metallic nanoparticles have numerous uses in the biomedical field, including bio-imaging, drug delivery, cancer treatment, medical diagnosis, catalytic activity, sensors, and more.

ACKNOWLEDGMENTS

The authors thank Wollega University for providing the necessary support.

DECLARATION

The authors declare that there is no conflict of interest.

DATA AVAILABILITY STATEMENT

Lema, Y & Asnake, L. *Sci. Technol. Arts Res. J., Oct.-Dec. 2023, 12(4), 129-146* The necessary data are available within the article materials.

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