



UNIVERSITY OF VENDA
SCHOOL OF ENVIRONMENTAL SCIENCES
DEPARTMENT OF HYDROLOGY AND WATER RESOURCES

A Study into the Interaction of Gold nanoparticles Released into Municipal
Drinking water and Wastewater systems

Student Name: Shumani Alfred Raedani

Student Number: 11531994

Signed (Supervisor):



.....Date:18/01/2016.....

Dr JR Gumbo



17/01/2016

Signed (Co-Supervisor):

.....Date:

Prof WM Gitari



Signed (Co-Supervisor):Date: January 18, 2016.....

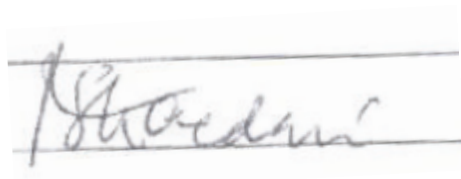
Dr Frankline K. Keter

**A Dissertation submitted to the Department of Hydrology and Water Resources,
University of Venda, for the fulfilment of the requirements for the degree of
Master of Environmental Science in Hydrology and Water Resources**

Januray 2016

Declaration

I, **Shumani Alfred Raedani**, do hereby declare that this dissertation project submitted to the University of Venda for the fulfilment of Masters' Degree in the Department of Hydrology and Water Resources, School of Environmental Sciences, is my own independent work. This work has not been submitted before to any institution by me, or any other person in fulfilment of requirements for any qualification.



Signature

.....18/01/2016.....

Date

Acknowledgement

I would like to thank first of all Dr J.R Gumbo for his overwhelming support during this project. His guidance is well appreciated. I would also like to pass my sincere gratitude to Professor WM Gitari and Dr Frankline Keter (Mintek) for their technical and material support.

My family and friends have always been around me for support. I would like to highly acknowledge the financial assistance provided by National Research Foundation (NRF) and the University of Venda to undertake this research.

Finally, God's grace has always been upon me

List of abbreviations and acronyms

Au	gold
Au-NPs	gold nanoparticles
NPs	nanoparticles
ENPs	engineered nanoparticles
COD	chemical oxygen demand
NOM	natural organic matter
Cu	copper
Si	Silicon
Co	Cobalt
Mg	Magnesium
Fe	Iron
DBPs	disinfection byproducts
Zp	Zeta potential
DLS	Dynamic light scattering
EDX	Energy dispersive x-ray

Abstract

This research involves the investigation of the interaction of different sized Nano Gold particles released into municipal drinking water and municipal waste water. Waste water was collected from Malamulele waste water treatment plant and the municipal water was collected at Mintek in Johannesburg, Randburg, South Africa. The waste water was analysed using ICP-MS to detect the metals and anions in it. The results showed the abundance of Sulphur (464 ppm), Calcium (28 ppm), Chloride (27.8 ppm), Iron (20 ppm), Magnesium (8.2 ppm), silicon (6.192 ppm) in descending order and other trace elements, including gold, that were immeasurable (<0.1). The simulated situation was created by adding 20nm gold and 40nm gold nanoparticles into municipal drinking water and waste water and kept at different environmental conditions (light, light and agitation, dark, dark and agitation) under aerobic and anaerobic conditions over a period of two months. Physico-chemical properties (pH and chemical oxygen demand) of the solutions were checked once in a month. The pH fluctuated between the acceptable ranges (5.5 – 9.5) for the two month period. Both municipal water and waste water, with and without gold nanoparticles, under aerobic condition showed an increase in chemical oxygen demand. The gold content in waste water under anaerobic condition showed an increase while under aerobic condition the decline in gold content was evident. The zeta potential of gold nanoparticles in waste water in light and agitation showed (-30 mV) while waste water on other environmental condition (light, dark and dark with agitation) presenting unstable (-18 mV) charge, but the charge shifted positively on the second month rendering them also unstable. Dynamic light scattering and TEM were used to check any possible aggregation or agglomeration of nanoparticles in the waste water. There were some few discrepancies where TEM and DLS contradict, but overall there was no significant probability of any aggregation of gold nanoparticles. The EDX was used to confirm the presence of Au^0 in the waste water (with added gold nanoparticles). The research did show that the gold nanoparticles would exist as Au^0 in the waste water and thus the discharge of Au-NPs to the sewer system is not recommended, but rather recycle them.

Key words: Gold (Au), Gold nanoparticles (Au-NPs), Nanomaterials, NanoGold, Nanoproducts, Nanostructures, Nanotechnology.

List of Figures

Figure 1: The initial mean pH of the water samples and gold nanoparticles before mixing, whiskers reflect standard deviation of triplicates	20
Figure 2: pH variations of (a) municipal water and (b) waste water with and without gold nanoparticles (Au-NPs) in light conditions.	21
Figure 3: pH variation of municipal drinking water with (zero Au-NPs, 20nm Au-NPs, 40nm Au-NPs, a mixture of 20nm and 40nm Au-NPs) in light and agitation condition.....	25
Figure 4 : pH variation of municipal waste water with (zero Au-NPs, 20nm Au-NPs, 40nm Au-NPs, a mixture of 20nm and 40nm Au-NPs) in light and agitation condition.....	26
Figure 5: pH variation of municipal drinking water with (zero Au-NPs, 20nm Au-NPs, 40nm Au-NPs, a mixture of 20nm and 40nm Au-NPs) in dark and agitation condition.....	27
Figure 6: pH variation of municipal waste water with (zero Au-NPs, 20nm Au-NPs, 40nm Au-NPs, a mixture of 20nm and 40nm Au-NPs) in dark and agitation condition.....	28
Figure 7: pH variations under anaerobic conditions of (a) municipal waste water with a mixture of 20nm and 40nm Au-NPs in light and agitation, a mixture of 20nm and 40nm Au-NPs in dark and agitation, 40nm Au-NPs in light and lastly 40nm Au-NPs in dark (b) municipal water with a mixture of 20nm and 40nm Au-NPs in light and agitation, a mixture of 20nm and 40nm Au-NPs in dark and agitation, 40nm Au-NPs in light and lastly 40nm Au-NPs in dark.....	29
Figure 8: Chemical oxygen demand (COD) of (a) municipal water and (b) wastewater with (zero, 20nm Au-NPs, 40nm Au-NPs and a mixture of 20nm and 40nm Au-NPs) in light condition, no agitation	31
Figure 9: Chemical oxygen demand (COD) of (a) municipal water and (b) waste water with (zero, 20nm Au-NPs, 40nm Au-NPs and a mixture of 20nm and 40nm Au-NPs) in light and agitation condition.	33
Figure 10: Chemical oxygen demand (COD) of (a) municipal water and (b) waste water with (zero, 20nm Au-NPs, 40nm Au-NPs and a mixture of 20nm and 40nm Au-NPs) in dark (away from direct light) condition	34
Figure 11: Chemical oxygen demand (COD) of municipal water (a) and waste water (b) with (zero, 20nm Au-NPs, 40nm Au-NPs and a mixture of 20nm and 40nm Au-NPs) in dark and agitation condition	35
Figure 12: (a) Municipal water with a mixture of 20nm and 40nm Au-NPs under light and agitation and dark and agitation and water with 40nm Au-NPs in light and also in dark all under anaerobic condition. (b) COD variations of waste water with a mixture of 20 and 40nm Au-NPs in light/dark with agitation and waste water with 40nm Au-NPs in light/dark all under anaerobic condition.....	36

Figure 13: (a) Zeta potential of municipal water with zero, 20nm, 40nm and a mixture of 20nm and 40nm Au-NPs. (b) Zeta potential of waste water with zero, 20nm, 40nm and a mixture of 20nm and 40nm Au-NPs under aerobic and light conditions (no agitation) for two months period. 41

Figure 14: (a) Zeta potential from municipal water with zero, 20nm, 40nm and a mixture of 20nm and 40nm Au-NPs. (b) Zeta potential from waste water with zero, 20nm, 40nm and a mixture of 20nm and 40nm Au-NPs under aerobic and light conditions with agitation for two months period. 43

Figure 15: (a) Zeta potential from municipal water with zero, 20nm, 40nm and a mixture of 20nm and 40nm Au-NPs. (b) Zeta potential from waste water with zero, 20nm, 40nm and a mixture of 20nm and 40nm Au-NPs under aerobic and dark conditions (no agitation) for two months period. 45

Figure 16: (a) Zeta potential from municipal water with zero, 20nm, 40nm and a mixture of 20nm and 40nm Au-NPs. (b) Zeta potential from waste water with zero, 20nm, 40nm and a mixture of 20nm and 40nm Au-NPs under aerobic, dark conditions with agitation for two months period. 47

Figure 17: (a) Zeta potential from municipal water with a mixture of 20nm and 40nm Au-NPs in light and agitation, a mixture of 20nm and 40nm Au-NPs in dark and agitation, 40nm Au-NPs in light and lastly 40nm Au-NPs in dark. All samples kept under anaerobic condition. (b) Zeta potential from waste water with a mixture of 20nm and 40nm Au-NPs in light/dark and agitation, a mixture of 20nm and 40nm Au-NPs in dark and agitation, 40nm Au-NPs in light and 40nm Au-NPs in dark. All samples kept under anaerobic condition. 49

Figure 18: Dynamic light scattering under aerobic, light (no agitation) conditions of: (a) waste water with zero, 20nm, 40nm and a mixture of 20nm and 40nm Au-NPs. (b) Municipal drinking water with zero, 20nm, 40nm and a mixture of 20nm and 40nm Au-NPs. 55

Figure 19: Dynamic light scattering under aerobic, light and agitation conditions of: (a) municipal waste water with zero, 20nm, 40nm and a mixture of 20nm and 40nm Au-NPs. (b) Municipal drinking water with zero, 20nm, 40nm and a mixture of 20nm and 40nm Au-NPs. 60

Figure 20: Dynamic light scattering under aerobic, dark (no agitation) conditions of: (a) municipal waste water with zero, 20nm, 40nm and a mixture of 20nm and 40nm Au-NPs. (b) Municipal drinking water with zero, 20nm, 40nm and a mixture of 20nm and 40nm Au-NPs. 64

Figure 21: Dynamic light scattering under aerobic, dark conditions with agitation of: (a) municipal waste water with zero, 20nm, 40nm and a mixture of 20nm and 40nm Au-NPs. (b) Municipal drinking water with zero, 20nm, 40nm and a mixture of 20nm and 40nm Au-NPs. 69

Figure 22: Dynamic light scattering under anaerobic of (a)municipal waste water with a mixture of 20nm and 40nm in light and agitation, municipal waste water with a mixture of 20nm and 40nm in dark and agitation, waste water with 40nm Au-NPs in light and waste water with 40nm Au-NPs in dark. (b)

Municipal drinking water with a mixture of 20nm and 40nm in light and agitation, municipal drinking water with a mixture of 20nm and 40nm in dark and agitation, municipal drinking water with 40nm Au-NPs in light and municipal drinking water with 40nm Au-NPs in dark 73

Figure 23: EDX micrograph for waste water with 40nm Au-NPs kept in light for one month (W40L) 82

Figure 24: EDX micrograph for waste water with a mixture of 20nm and 40nm Au-NPs kept in light and agitation for one month (WMLA)..... 83

Figure 25: EDX micrograph for waste water with a mixture of 20nm and 40nm Au-NPs kept in dark and agitation for one month (WMDA) 83

Figure 26: EDX micrograph for municipal water with a mixture of 20nm and 40nm Au-NPs kept in dark and agitation for one month (CMLA) 84

List of Pictures

Picture 1:(a)Municipal waste water with and without Au-NPs first day of preparation and were kept under light environment. (b): Municipal waste water with and without Au-NPs kept in light for 1 month. From left to right: waste water with no Au-NPs, waste water with 20nmAu-NPs, waste water with 40nm Au-NPs and waste with a mixture of 20nm and 40nm Au-NPs. (c) municipal waste water with and without Au-NPs in light after 2 months. 1d. municipal waste water with (zero, 20nm, 40nm and a mixture of 20nm and 40nm) Au-NPs after one month under light and agitation condition	24
Picture 2: municipal waste water mixed with 40nm Au-NPs kept in light for one month (W40L R2).....	56
Picture 3: Municipal waste water mixed with 40nm Au-NPs in light for a period of two months (W40LR3).....	56
Picture 4: Municipal drinking water with 40nm Au-NPs in light for a month (C40LR2).....	58
Picture 5: Municipal drinking water with a mixture of 20nm and 40nm Au-NPs in light after two months (CMLR3)	59
Picture 6: Municipal drinking water with a mixture of 20nm and 40nm Au-NPs kept in light and agitation for two months (CMLA aero R3)	61
Picture 7: municipal waste water with 40nm Au-NPs in light and agitation for one month (W40LAR2) .	62
Picture 8: municipal waste water with 40nm Au-NPs kept in light and agitation the two month period (W40LAR3).....	63
Picture 9: TEM picture of municipal drinking water mixed with 40nm Au-NPs kept under dark conditions for one month (C40DR2)	65
Picture 10: Waste water with 40nm Au-NPs away from light (W40D).....	66
Picture 11: TEM picture of municipal drinking water mixed with 40nm Au-NPs kept under dark conditions for two months (C40DR3).....	67
Picture 12: The TEM picture of waste water with a mixture of 20 and 40nm Au-NPs kept away from light for a month (WMDR2)	68
Picture 13: Waste water with 20nm Au-NPs under aerobic dark and agitation condition after two months of incubation (W20DAR2)	70
Picture 14: Municipal drinking water with a mixture of 20nm and 40nm Au-NPs on agitation away from light for a month (CMDAR2)	71
Picture 15: Municipal waste water with a mixture of 20nm and 40nm Au-NPs under anaerobic condition in dark and agitation for two months (WMDA anaerobic R2)	72

Picture 16: Municipal water was mixed with 40nm Au-NPs under anaerobic condition in light for a month (C40L anaerobic R2).....	74
Picture 17: Waste water with 40nm Au-NPs in light under anaerobic condition kept for two months (W40L anaerobic R3)	74
Picture 18: municipal waste water with 40nm Au-NPs in dark under anaerobic condition kept for one month (W40D anaerobic R2).....	75
Picture 19: municipal waste water with 40nm Au-NPs in dark under anaerobic condition for two months (W40D anaerobic R3).....	76
Picture 20: Municipal waste water with a mixture of 20nm and 40nm Au-NPs under anaerobic condition with agitation and kept away from light for one month (WMDA anaerobic R2).....	77
Picture 21: Municipal water with a mixture of 20nm and 40nm Au-NPs under anaerobic condition in light and agitation after one month (CMLA anaerobic R2)	77
Picture 22: Municipal drinking water with a mixture of 20nm and 40nm Au-NPs under anaerobic condition in light and agitation after two months (CMLA anaerobic R3).....	78
ht environment. 1b: Municipal waste water with and without Au-NPs kept in light for 1 month. From left to right: waste water with no Au-NPs, waste water with 20nm Au-NPs, waste water with 40nm Au-NPs and waste with a mixture of 20nm and 40nm Au-NPs. 1c: municipal waste water with and without Au-NPs in light after 2 months. 1d. municipal waste water with (zero, 20nm, 40nm and a mixture of 20nm and 40nm) Au-NPs after one month under light and agitation condition	24
Picture 2: municipal waste water mixed with 40nm Au-NPs kept in light for one month (W40L R2).....	56
Picture 3: Municipal waste water mixed with 40nm Au-NPs in light for a period of two months (W40LR3).....	56
Picture 4: Municipal drinking water with 40nm Au-NPs in light for a month (C40LR2).....	58
Picture 5: Municipal drinking water with a mixture of 20nm and 40nm Au-NPs in light after two months (CMLR3)	59
Picture 6: Municipal drinking water with a mixture of 20nm and 40nm Au-NPs kept in light and agitation for two months (CMLA aero R3)	61
Picture 7: municipal waste water with 40nm Au-NPs in light and agitation for one month (W40LAR2) .	62
Picture 8: municipal waste water with 40nm Au-NPs kept in light and agitation the two month period (W40LAR3).....	63
Picture 9: TEM picture of municipal drinking water mixed with 40nm Au-NPs kept under dark conditions for one month (C40DR2)	65

Picture 10: Waste water with 40nm Au-NPs away from light (W40D).....	66
Picture 11: TEM picture of municipal drinking water mixed with 40nm Au-NPs kept under dark conditions for two months (C40DR3).....	67
Picture 12: The TEM picture of waste water with a mixture of 20nm and 40nm Au-NPs kept away from light for a month (WMDR2).....	68
Picture 13: Waste water with 20nm Au-NPs under aerobic dark and agitation condition after two months of incubation (W20DAR2).....	70
Picture 14: Municipal drinking water with a mixture of 20nm and 40nm Au-NPs on agitation away from light for a month (CMDAR2).....	71
Picture 15: Municipal waste water with a mixture of 20nm and 40nm Au-NPs under anaerobic condition in dark and agitation for two months (WMDA anaerobic R2).....	72
Picture 16: Municipal water was mixed with 40nm Au-NPs under anaerobic condition in light for a month (C40L anaerobic R2).....	74
Picture 17: Waste water with 40nm Au-NPs in light under anaerobic condition kept for two months (W40L anaerobic R3).....	74
Picture 18: municipal waste water with 40nm Au-NPs in dark under anaerobic condition kept for one month (W40D anaerobic R2).....	75
Picture 19: municipal waste water with 40nm Au-NPs in dark under anaerobic condition for two months (W40D anaerobic R3).....	76
Picture 20: Municipal waste water with a mixture of 20nm and 40nm Au-NPs under anaerobic condition with agitation and kept away from light for one month (WMDA anaerobic R2).....	77
Picture 21: Municipal water with a mixture of 20nm and 40nm Au-NPs under anaerobic condition in light and agitation after one month (CMLA anaerobic R2).....	77
Picture 22: Municipal drinking water with a mixture of 20nm and 40nm Au-NPs under anaerobic condition in light and agitation after two months (CMLA anaerobic R3).....	78

List of Tables

Table 1: The ICP MS operating conditions	18
Table 2: Concentrations of metals in municipal waste water sample	19
Table 33: Places where peaks are visible on the EDX graphs	81

List of Maps

Map 1: Malamulele area in Limpopo, South Africa (22.9982° S, 30.6961° E).....	4
Map 2: Mintek map in Johannesburg South Africa(S 26° 2' 26.66" E 27° 57' 8.73")	5

Table of Contents

Declaration.....	ii
Acknowledgement	iii
List of abbreviations and acronyms	iv
Abstract.....	v
List of Figures	vi
List of Pictures.....	ix
List of Tables	xii
List of Maps.....	xii
Chapter 1: Introduction	1
1.1 Background to the Research	1
1.3 Motivation.....	3
1.4 Research Objectives.....	4
1.4.1 Specific Objectives:	4
1.5 The Study Area	4
Chapter 2: Literature Review	6
2.1 Nanotechnology	6
2.2. Nanoproducts	6
2.2.1Gold nanomaterials and their reactivity	7
2.3 Environmental Fate and Transport.....	8
2.3.1. Nanomaterials in Aquatic Systems	9
2.4 Potential Releases, Human Exposure and impacts.....	10
2.5 Nanoparticles are Emerging Contaminants.....	12
2.6 Interaction of nanoparticles and various contaminants in waste water.....	12
2.7. Impact of nanomaterials on COD in waste water	13
Chapter 3: Materials and Methods.....	14
3.1 Preparation of gold nanoparticles (Au-NPs).....	14

3.2 Sample collection and preparation	14
3.2.1. Waste water	14
3.2.2. Municipal tap water	14
3.2.3. Storage of samples	15
3.3 Samples for analysis	15
Chapter 4: The Stability Investigations of Gold nanoparticles in aqueous phases	16
Abstract	16
4.1 Introduction	16
4.2 Materials and Methods	16
4.2.1 Analysis of physical chemical parameters	16
4.2.2 Analysis of metals and non-metals including Au concentration	17
4.2.3 Data analysis	18
4.3 Results and discussion	19
4.3.1 The metals and non-metals in the municipal waste water	19
4.3.2 The pH stability of gold nanoparticles during their transition from light to dark conditions	19
4.3.3 The interaction of gold nanoparticles and organic matter (chemical oxygen demanding substances) during their transition from light to dark conditions	30
4.4 Conclusion	38
Chapter 5: The Hydrodynamic diameter of Gold nanoparticle (Au-NPs) in the different water environments	39
Abstract	39
5.1 Introduction	39
5.2 Materials and Methods	39
5.2.1 Zeta potential (Zp)	39
5.2.2 Data analysis	40
5.3 Results and discussion	40
5.4 Conclusion	50

Chapter 6: The Size and Morphology of Gold nanoparticles using Dynamic Light Scattering (DLS) and Transmission electron Microscopy (TEM) in Aqueous phases	52
Abstract.....	52
6.1 Introduction	52
6.2 Materials and Methods.....	53
6.2.1 Dynamic light scattering (DLS)	53
6.2.2 Transmission electron microscope (TEM) and EDX.....	53
6.2.3 Data analysis	53
6.3 Results and discussion	54
6.4 Conclusion	79
Chapter 7: The presence of Au in NanoGold particles.....	80
Abstract.....	80
7.1 Introduction	80
7.2 Materials and Methods.....	80
7.2.1 Transmission electron microscope (TEM) and EDX	80
7.2.2 Data analysis	81
7.3 Results and discussion	81
7.4 Conclusion	84
Chapter 8: Conclusion and recommendations	85
8.1 Conclusion	85
8.2 Recommendations	86
References	87

Chapter 1: Introduction

1.1 Background to the Research

Pure gold, yellow in colour, can appear red, blue, green or brown when reduced to a nanometer scale. This colour change is as a result of the interaction of conduction band electrons in the metallic nanoparticles with an electric field of the incident light (Alkilany and Murphy, 2010). Few gold compounds occur naturally in the environment (Gonzalez-Anaya et al., 2011). Gold nanomaterials have physico-chemical properties that continue to draw a lot of interest to a variety of industries resulting in these materials being included in a range of consumer products and manufacturing systems. These include optical, electric and biocompatible properties. Furthermore, gold nanoparticles are attractive in diagnostics, sensors, therapeutics, catalysts and nanoelectronics applications all of which are meant to address societal challenges (Trudel, 2011).

Metal nanoparticles surfaces can be altered when treated with different organic compounds/ligands. These alterations of nanoparticles have gained an extensive consideration because of their potential application in catalysis, sensors and optical materials and in the development of biological tracers and optoelectronic nanodevices. As such, nanotechnology can be used to engineer and fabricate new materials with improved qualities. At nanometer scale, metals exhibits high surface area per volume ratio which leads to materials exhibiting properties that are different from their bulk counterparts. Properties including greater catalytic efficiency, increased electrical conductivity, and improved hardness and strength are a result of the reduction in size of materials (USEPA, 2007). These properties make engineered nanoparticles (ENPs) very reactive and useful in many applications (Michelle, 2013).

It is noteworthy that nanotechnology promotes efficiency and effective use of materials. For example, nanotechnology may improve the functionality of catalytic converters while the mass of platinum used is reduced by up to 95% to the amount of metal required (USEPA, 2007).

Depending on the source, nanoparticles are divided into two categories. Firstly, there are the natural nanoscale particles which are within the broad range of naturally occurring particulates that are of 1 to 100nm in size, like particulates from forest fire and biological particulate. Secondly, there are engineered nanoscale particles. These are, in contrast with natural ones, artificially synthesized. The properties and other parameters are fabricated in order to make them better suited for the purpose for which they are synthesized.

However, the use of these nanomaterials poses a great challenge to mankind and the environment. In the latter case, little is known of the impact of nanomaterials disposed into the waste-water system or sewage system. For instance, most materials at nanoscale display properties that are different from those in bulk state. This is the reason why some nanoparticles can be toxic even though in their bulk forms are non-poisonous. Nanoparticles have the capability to absorb other toxic substances thereby becoming more toxic. They may also transform into toxic materials with the ability to move further through air or water (Schutte and Focke, 2007).

In the case of South Africa, the water resource is under pressure in terms of quality and quantity (Schutte and Focke, 2007). In addition, the climate change due to global warming is likely to put more stress on the resource. As such there has to be concerted efforts to ensure minimal pollution of the waste water systems, e.g. rivers and dams. This is done in two ways; (i) ensure correct waste disposal approaches to minimize their impact and (ii) refining the waste treatment processes to ensure efficiency. In the latter case, wastewater is treated to remove all the pollutants before released into water resources like rivers. Disposal of raw or inadequately treated sewage into the environment is the main source of pathogens and other pollutants in the water environment. This brings the question of whether the wastewater treatment processes in existence are efficient in removing all contaminants including metal nanoparticles (Wen et al., 2009). This needs to be addressed considering that water is a scarce commodity, and the existing water treatment processes do not cover nanomaterials.

1.2 Statement of the Problem

The use of nanomaterials and nanoproducts is increasing rapidly in society. For instance, nanomaterials are now incorporated in consumer products such as soaps, sunscreens and toothpastes. The remnants of these products end up being disposed into the wastewater systems, air and soil yet very little is known about their interaction with the microbes in the wastewater which are the integral part of the biological treatment process of wastewater.

1.3 Motivation

The ability of nanoparticles properties to change and differ from that of their bulk materials makes nanoparticles susceptible to exploitation for many industrial applications. Some studies regard nanoparticles as non-hazardous, while other studies report some toxic effects (Abdelhalim et al., 2012). With this kind of information, there is a need for concerted effort to investigate the potential risks that nanomaterials pose, especially in the wastewater systems. This is because increase in accumulation of NanoGold particles in wastewater, groundwater and soil can pose a potential risk to the environment, human beings and aquatic population. For example there is a need to investigate the impact nanomaterials have on the microbial system in a waste-water treatment plant such as nitrifying bacteria that are key in waste treatment. Given that there is a wide range of nanomaterials of different formulations, shapes and sizes, the purpose of this study was to focus on spherical gold nanoparticles which are among the most used type of nanomaterials.

For the purpose of this study, a mixture and or singular gold nanoparticles were added to municipal drinking water and municipal wastewater in order to understand the interactions and behaviour of gold nanoparticles in their passage in municipal drinking water to sewers (municipal wastewater). The study was undertaken under aerobic and light conditions, simulating the surface interaction with municipal drinking water and under anaerobic and dark conditions, thus simulating the sewers.

1.4 Research Objectives

In view of the fact that there is little information on the effect of nanomaterials on the environment, especially waste water system, largely due to the fact that it is an emerging field of study, the main objective of the study is to predict the interactions that occur among a mix of gold nanoparticles with waste-water constituencies under aerobic and anaerobic conditions.

1.4.1 Specific Objectives:

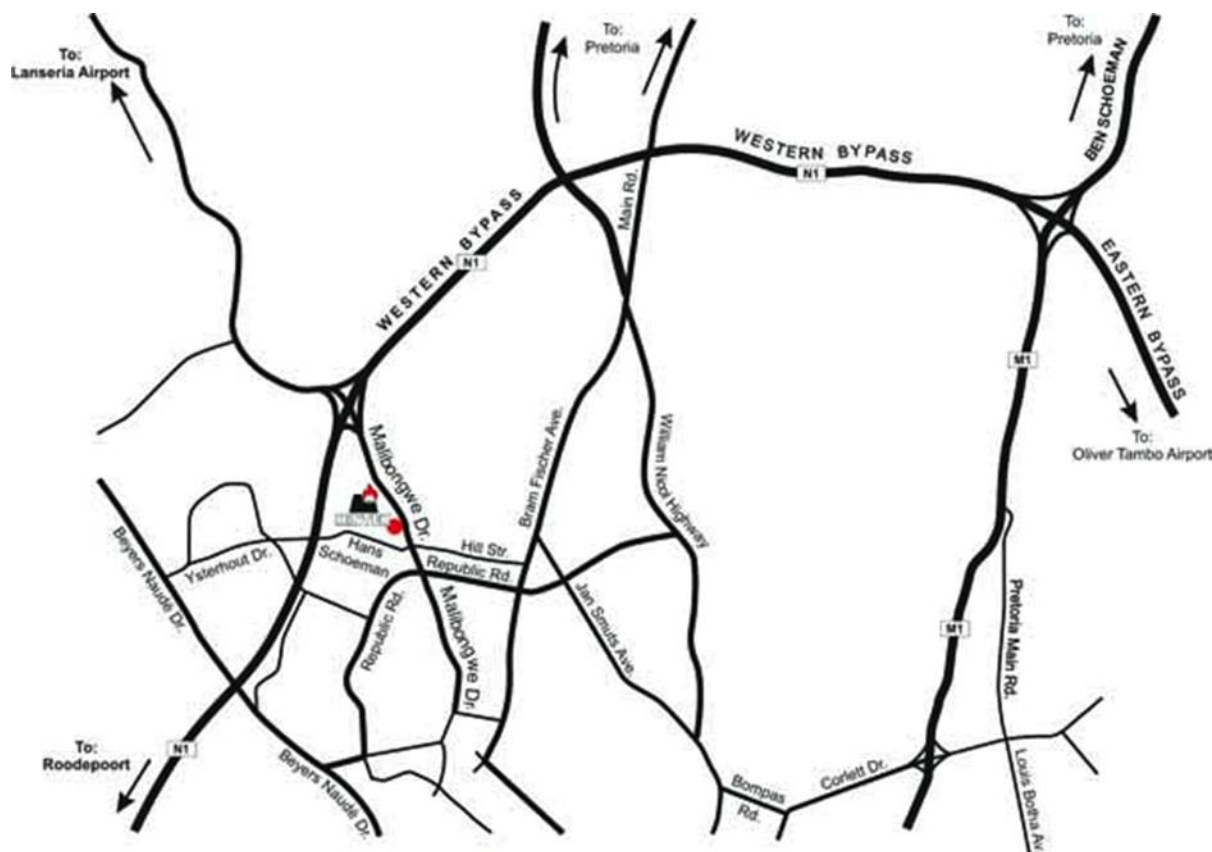
Specific objectives for the study would include the following

- To investigate the stability of Gold nanoparticles in aqueous phases (municipal drinking and waste waters);
- To determine the hydrodynamic diameter of Gold nanoparticle (Au-NPs) in aqueous phases (municipal drinking and waste waters);
- To determine the size and morphology of Gold nanoparticles in aqueous phases (municipal drinking and waste waters) and
- To investigate the presence of Au in NanoGold particles in aqueous phases (municipal drinking and waste waters);

1.5 The Study Area



Map 1: Malamulele area in Limpopo, South Africa (22.9982° S, 30.6961° E)



Map 2: Mintek map in Johannesburg South Africa(S 26° 2' 26.66" E 27° 57' 8.73")

The research study was carried out at the Advanced Materials Division (AMD) laboratories of Mintek Randburg, Johannesburg. The municipal drinking water was collected from the Johannesburg Water (Mintek tap water) and the municipal waste water was collected from Malamulele wastewater treatment plant, Limpopo.

Chapter 2: Literature Review

2.1 Nanotechnology

Nanotechnology is the art of manipulating matter at nanoscale to produce nanomaterials and study these materials of dimensions usually between 1 and 100nm (Handy and Shaw, 2007). Nanoparticles are materials with nanoscale sizes that exhibit properties that are different from bulk samples of the same material (Auffan et al., 2009; Abdelhalim et al., 2012). According to Rastogi (2011) nanotechnology is a highly multidisciplinary field of applied sciences involved in the design and application of nanoparticles for various purposes. Nanoparticles have many advantages over their bulk analogues, and are attributed to their unique physical and chemical properties arising from their small size, such as high surface area to volume ratio.

Nanotechnology is currently employed to fabricate and engineer domestic products such as soaps and toothpastes and some nanomaterials are used as additives to paints, clothing, food containers and bandages (Hou et al., 2012). Like many other products that were manufactured in the past, they could turn to cause unexpected harm to the environment and human health. As such, research on how these nanoproducts and nanomaterials are used and released into the wastewater and the environment is necessary. The outcomes can be used to carefully estimate nanomaterials toxicities and anticipate how easily they progress in the water, air and soil. Communities can be cautioned to work with materials that are useful, safe and avoid the hazardous ones (Schmidt, 2007).

2.2. Nanoproducts

Nanoproducts are used in many industries including electronics, optics, textiles, medical devices, biosensors and in environmental rehabilitation (Handy and Shaw, 2007). They are also used as high extinction dyes for colorimetric sensing of anions in water (Madu et al., 2011). The Nanotechnology Consumer product Inventory (CPI) listed 1012, which increased to 1814 in 2015, products embedded with nanoparticles from 409 companies in 24 countries. Even though

most of the products do not advertise their nanomaterial contents, of the listed, health and fitness was found to have the largest listing of products. These include personal care products such as tooth brushes, lotions, hairstyling tools and body persperants products (Vance et al., 2015). The product after use are disposed when the person takes a bath and nanoparticles then enter the sewer system and is main source of sewage contamination.

Currently, it is not clear how nanoproducts disposed into the environment behave. Some materials are released during processing, use or disposal; either intentionally or unintentionally. More so, the rates at which these materials are released do vary. The behaviour of nanomaterials that have been disposed after use is also not yet clear. For instance, the information on how they react with each other and other materials in the wastewater or soil is uncertain (Karn and Aguar, 2007).

2.2.1 Gold nanomaterials and their reactivity

The discovery of gold nanoparticles (Au-NPs) has changed the gold chemistry. An example is the dark red colour of solutions of Au-NPs, which is due to the surface plasmon resonance of the nanoparticles. The common Au-NPs usually range between 10 – 100 nanometers (nm) with specific surface area in the $1 - 3\text{m}^2\text{g}^{-1}$ range. Colloidal gold property is the most exploited form of gold nanotechnology. It was used as early as the fourth century to decorate, make stained glass and as pigment in the decoration of porcelain (Trudel, 2011). Au-NPs incorporating other materials can be very effective as catalysts. For example, Au-NPs covered with palladium are very effective catalysts for removing tri-chloroethane (TCE) from groundwater 2,200 times better than palladium alone (Tiwari et al., 2008).

The use of gold nanoparticles (Au-NPs) for detecting and treating cancer is a new and exciting field of research. Currently, methods of diagnosis and treatment of cancer are expensive and can also be harmful to the body. Au-NPs, however, offer a potentially affordable approach of targeting cancerous cells (Abdelhalim et al., 2012). Gold particles coated with specific antibodies can be used to detect the presence and position of antigens on the surfaces of cells. Au-NPs are mostly used because of their ease of synthesis, stability in suspension and unique optical properties (Glenn et al., 2012). These unique optical properties of NanoGold have been explored

for ultra-sensitive detection, while its surface-modifiability has been explored for selectivity. Au-NPs enhance the dynamic light scattering and thus improve the sensitivity of optical based biosensors using surface-enhanced Raman and scattering as sensing method (Michelle, 2013).

Most bulk metal materials are highly unreactive; however in their nanoscale form they exhibit interesting physico-chemical properties. Apart from the nanoscale size, other factors that affect reactivity and interaction of particles with the environment include particle size and shape (Roux, 2008). Alkilany and Murphy (2010) found that gold nanoparticles less than 2 nm in diameter showed evidence of chemical reactivity that do not occur at larger sizes. This is attributed to the large surface area to volume ratio of nanomaterials. For example, bulk gold hardly reacts with substances around it, but Au-NPs can attach to cell membranes destroying bacteria and living cells (Schutte and Focke, 2007; Elsaesser and Howard, 2012).

There is a number of studies reported looking at the interaction of nanomaterials with cells. One case in point is the work by Ray et al. (2008) which showed that cationic particles were generally toxic at much lower concentrations than their anionic counterparts. This toxicity was associated with the electrostatic interaction between the cationic nanoparticles and the negatively charged cell membranes (Alkilany and Murphy, 2010), which allows nanoparticles to cross the cell membrane easily. In spite of all the advantages of this class of compounds, their risk profiles to human and environment remain largely unknown and the need for such evaluation is as important as the advantages that ensue from these nanomaterials.

2.3 Environmental Fate and Transport

As more communities are beginning to use nanoproducts in large quantities; there is an increased possibility for environmental exposure to accumulated nanoparticles. Nanoparticles are released or disposed into the environment either directly or indirectly. For instance, nanoparticles can be released directly during manufacturing or processing of nanomaterial-based products and disposal of nano-waste from industrial process, or indirectly by consumer waste disposal of products with nanocomponents that dissipate into the environment slowly over time.

There is no doubt that research work needs to be done to determine whether nanomaterials released into the environment, especially waste-water, retain their nanoscale sizes, original structure and reactivity in the environment (Ray et al., 2008), and if so, what are the implications involved?

2.3.1. Nanomaterials in Aquatic Systems

The behaviour of nanomaterials in aquatic environments depends on aqueous solubility or dispersability, interactions between nanomaterial and natural and anthropogenic chemicals in the system, and biological and abiotic processes. Nanoparticles normally settle more slowly than larger particles of the same material.

Granted, nanotechnology can lead to economical and high performance water treatment systems. It has the capacity and performance potential to generate technically and environmentally appropriate solutions to water related problems over a wide spectrum such as, nano-based water filters which possess antibacterial properties because of silver nanoparticles. In addition to improved treatment technologies, nanotechnology offers the promise of cleaning up historic pollution problems. Nanotechnology has the potential for instant and continuous monitoring of water quality; however its biggest impact on the environment could be in pollution prevention through improved clean technologies for better conservation of materials and elimination of waste production. Unlike other contaminants, e.g. heavy metals and other inorganic substances, nanomaterials are not sufficiently removed by standard treatment processes from wastewater and may not be effectively removed from drinking water treatment (Schutte and Focke, 2007). This is one of the major concerns pertaining to nanotechnology.

Given that the majority of the nanomaterials are engineered, their properties such as aggregation influence the transformation and indeed the resultant toxicity or cytotoxicity profiles. This presents a huge challenge to society with respect to waste water management systems and indeed aquatic environments considering that the current processes are not tailor-made for handling nanowastes.

Some nanoparticles such as TiO_2 showed toxicity to aquatic organisms, both unicellular (e.g. bacteria or protozoa) and animals such as daphnia fish (Bhattacharice et al., 2013). The size and shape of nanoparticles were found to influence the interaction between particles and cells. The presence of light was found to be a significant factor in increasing toxicity in aquatic environment. Exposure of metal nanoparticles to the aquatic environment has been found to be detrimental to aquatic organisms (Bhattacharice et al., 2013).

2.4 Potential Releases, Human Exposure and impacts

Nanoproducts are released into the environment (water, soil and air) mainly through waste disposal. As for exposure to nanoparticles, the focus has been primarily on inhalation as the primary route of exposure. However, other exposure routes such as dermal, ocular, and oral, may also be found to be significant in humans depending on the scale of application of nanomaterials.

Free nanoparticles are a greater potential risk than those fixed within a coating. When nanoparticles enter the human body through inhalation, ingestion, or adsorption through the skin, they are able to move around and penetrate cells more easily than larger particles (Schutte and Focke, 2007).

Due to unique particle size, nanoparticles have a higher rate of distribution, modified particle morphology and particle composition, increased surface area, altered surface chemistry and particle reactivity in solution. All these modifications and alterations enable the nanoparticles to penetrate the human body through skin, inhaled into respiratory and gastrointestinal tract (Rastogi, 2012).

Some concentration levels of gold nanoproducts are believed to be inducers of oxidative stress as they cause a decrease in cellular calcium and zinc concentration (Abdelhalim et al., 2012). When size and electronic structure of nanoparticles are altered, the unusual catalytic properties turn up. When the sizes of gold nanoparticles are reduced to a few nanometers, they become extremely effective oxidation catalysts (Auffan et al., 2009). Some nanomaterials that enter animal tissues

may be able to pass through cell membranes or cross the blood-brain barrier. This may be a beneficial feature for transporting certain drugs and other treatments (USEPA, 2007).

Furthermore, most nanoparticles are able to stimulate the immune system and thus elicit inflammatory response through the secretion of various cytokines and other chemicals which is beneficial to mankind. Some nanoparticles are reported to have non-inflammogenic properties, which makes them favourable for usage in medical therapeutics (Rastogi 2012).

Be that as it may, nanoparticles have got a negative impact too as alluded before (*vide supra*). For instance, some studies have shown that nanoparticles in air could cause lung disease in animals (Handy and Shaw, 2007). Inhaled nanoparticles may be trapped in the lungs or be translocated, and the high biopersistence and reactivity of some nanomaterials raise issues of their fate in the environment. However, other factors that affect the possible risk of nanoparticles include the length of exposure, concentrations, particle size and the nature of the particle surface (Schutte and Focke, 2007).

Despite its uncertain environmental, health and safety impacts, nanotechnology has revealed an enormous potential for efficient and improved performance products for many commercial and industrial applications. There must be a concern to balance between avoiding environmental impacts of nanotechnology and the potential benefits gained from utilizing this technology. Measures taken to protect the environment from possible unfortunate effects caused by nanomaterials must also provide for the protection to people. It is also important not to inadvertently counterbalance some health gains in considering environmental improvement from the products themselves. However, society, as well as individuals, might accept the potential risks, if the benefits of nanotechnology such as the applications in cancer treatment and other areas of medicine and in more-efficient energy systems, are clear. One of the possible ways to provide these balances is to study the exposure of nanomaterials to humans in medicine and the advantages thereof as well as the impact on the environment. The latter part forms the major part of this study.

2.5 Nanoparticles are Emerging Contaminants

Production of nanomaterials and products containing nanomaterials is a fast growing field with many opportunities for improvement. According to Roux (2008), the most prevalent nanoparticles currently used in industries include metals such as silver and gold nanoparticles. These types of nanoparticles are most likely the future sources of environmental pollution. However, little scientific information is currently available on the fate of these nanomaterials in water and waste water treatment plants, whether they are present as biosolids or effluent, or their potential impact on the treatment processes (Schutte and Focke, 2007). Nanoparticles are not commonly measured in natural waters or in drinking water. The absence of technology in sewage treatment works specific for removal of nanomaterials in waste water is a cause for concern.

2.6 Interaction of nanoparticles and various contaminants in waste water.

Nanoparticles may be modified and transformed by interaction with the environment. These modifications may increase or decrease their ability to interact with cell membranes (Michelle, 2013). Transformation of nanomaterials influence the fate and behaviour of nanoparticles owing to nanostructure properties such as surface chemistry, aggregation, agglomeration, adsorption or absorption properties in addition to environmental factors such as pH, presence or absence of oxidants, complex ions, zeta potential, and effect of macromolecules and presence of other chemicals (Musee, 2010).

Due to their small size, high reactivity of nanomaterials will likely cause aggregation and agglomeration to form bigger particles (Musee, 2010). Nanoparticles can also bind to organic matter, metals and other contaminants in the waste water, which could lead to formation of lethal combinations. This needs to be investigated as well.

2.7. Impact of nanomaterials on COD in waste water

Chemical oxygen demand (COD) is the measure of the organic pollution in the wastewater. Microbes in the waste water are of great importance in the COD reduction and nitrogen removal. Thus, inhibition or negative impact on microorganisms responsible for these processes will compromise the biological treatment efficiencies of the waste water (Hou et al., 2011). Unlike Ag-NPs, which have been shown to have negative effect on COD and nitrogen removal by inhibiting the growth of nitrifying bacteria, little is known about the impact of gold nanoparticles in waste water systems (Kaegi et al., 2011).

In view of the above-discussed sections, it is clear that nanoproducts are fast becoming the norm in the society and the need to understand their environmental impact. This would help in adjusting the existing waste water treatment processes to cover new types of waste materials. As a start, this project will use gold nanoparticles as a case study, where their presence in the modelled waste water system was studied over a period of time.

Chapter 3: Materials and Methods

3.1 Preparation of gold nanoparticles (Au-NPs)

The Au-NPs (20 and 40nm) used in this project were produced and supplied by Mintek as a donation to the project. The Au-NPs concentration of 20nm was 46.2 ppm and 40nm was 27.2 ppm.

3.2 Sample collection and preparation

3.2.1. Waste water

About 20 litres of raw waste water were collected by dipping the container into Malamulele municipal waste water treatment plant from a primary stage of water treatment, and taken to the laboratory. The wastewater was distributed among several containers (1L bottles).

1. Bottle (B1) was a blank sample with just 190 ml of wastewater.
2. Bottle (B2) contained 190 ml waste water with 10 ml of 20nm gold nanoparticles added
3. Bottle (B3) contained 10 ml of 40nm Au-NPs in 190 ml of waste water.
4. Bottle (B4) contained of a mixture of a 5 ml of 20nm and 5 ml of 40nm Au-NP's in 190 ml of waste water.

3.2.2. Municipal tap water

The above procedure was repeated with Johannesburg Mintek tap water instead of waste water.

1. Bottle (B5) was a blank sample with just 190 ml of deionised water
2. Bottle (B6) contained 190 ml of tap water with 10 ml of 20nm gold nanoparticles added
3. Bottle (B7) contained 190 ml of tap water with 10 ml of 40nm Au-NPs added.
4. Bottle (B8) contained 190 ml of tap water with a mixture of a 5 ml of 20nm and 5 ml of 40nm Au-NP's.

3.2.3. Storage of samples

Another set of the test samples (B1 – B8) was prepared and stored in different lighting as well as agitating conditions as indicated below.

- Batch 1 (I) was placed on normal light
- Batch 2 (II) was placed in the dark
- Batch 3 (III) was placed on normal light with a magnetic stirrer for mixing
- Batch 4 (IV) was placed in the dark with a magnetic stirrer.

Samples were stored under these conditions for the period of two months.

3.3 Samples for analysis

Samples were analyzed on regular intervals for a period of two month with each of the methods that follows in the next Chapters. Ten (10) ml of gold nanoparticles were added to 190 ml of waste water or municipal water to give a total volume of 200 ml. A mixture of 20nm and 40nm Au-NPs was prepared as follows: 5 ml of 20nm was added with 5 ml of 40nm to make up 10 ml.

Chapter 4: The Stability Investigations of Gold nanoparticles in aqueous phases

Abstract

The stability of gold nanoparticles in the municipal drinking water and waste water was investigated. The study showed that the acidic gold nanoparticles were stable in wastewater and the acidic gold nanoparticles were changed to alkaline in municipal drinking water. Under anaerobic conditions, waste water and municipal drinking water in both light and dark with and without agitation showed an increase in pH in the second month of incubation. Waste water under aerobic condition showed a decrease in pH in the two months period. The interaction metal and non-metal content, sulphur (464 ppm), Calcium (28 ppm), Chlorides (27.8 ppm), Iron (20 ppm), Magnesium (8.2 ppm), Silicon (6.19 ppm) and trace elements, of the waste water was determined and these did not interfere with the stability of the gold nanoparticles. Chemical oxygen demand (COD) of waste water and municipal drinking water under anaerobic conditions showed consistency while there was a general increase in COD under aerobic conditions. The stability of gold nanoparticles was not affected by presence of organic compounds, as indicated by chemical oxygen demanding compounds.

4.1 Introduction

The physico-chemical properties: pH and chemical oxygen demand (COD), of waste water and municipal drinking water loaded with functionalised/stabilised gold nanoparticle (Au-NPs) were determined under light and dark conditions with and without agitation. The study was performed both under anaerobic and aerobic conditions.

4.2 Materials and Methods

4.2.1 Analysis of physical chemical parameters

The physico-chemical properties of nanoparticles present in tap and wastewater samples were analysed in triplicate for pH and chemical oxygen demand (COD) after calibration as per manufacturer (*Vide infra*).

The pH of the aliquot was checked using the Crison basic 2^o pH meter (Lasec, South Africa) at room temperature. The machine was calibrated with three standard solutions before use. Each

sample was homogenously mixed before the electrode was dipped into the solution. After each sample run, the pH meter electrode was rinsed thoroughly with high purity water. The electrode was wiped with dry paper towel to avoid cross-contamination and remove excess water. The pH readings and their relevant temperatures were recorded.

The COD of the samples was analysed in triplicate. All waste water samples were pre-diluted in a serial dilution up to 1 in 1000 with high purity water before analysis. Three (3) ml of the pre-diluted sample was transferred into the COD reagent. With a cap tightly closed, the solutions were vigorously mixed for 1 to 2 minutes. Solutions were then pre-heated at 148 °C for 2 hours in a Thermoreactor (TR) 620. The tests were left to cool for 10 minutes before they were mixed by swirling. The solution were then left to completely cool down for 30 minutes before analysed on the machine (Spectroquant Pharo 100) supplied by Merck. The results were recorded in triplicates.

4.2.2 Analysis of metals and non-metals including Au concentration

The analysis of metals, non-metals including Au concentration in the waste water sample were performed by ICP-MS (Kaegi et al., 2011) (supplied by Perkin Elmer, USA) with operating conditions on the Table 1 below.

Table 1: The ICP MS operating conditions

Description	Actual Value
Nebuliser Gas Flow	0.89 L/min
Auxiliary Gas Flow	1.4 L/min
Plasma Gas Flow	18.5 L/min
RF Power	1400 W
Deflector voltage	-9.5 V
AC rod offset	-15 V
Double charge: Ce^{++}/Ce^{+}	$\leq 0.03\%$
Oxide levels CeO^{+}/Ce^{+}	$\leq 2.5\%$
Sampler & Skimmer Cones	Nickel
Internal STD	^{187}Re
^{197}Au Calibration range	1 – 100 ppb

The municipal raw waste water (5 ml) of the thoroughly mixed samples was sent for ICP-MS analysis for metals and non-metals of interest in the waste water environment. Samples were allowed to settle before analysis as it was not possible to perform the test on a turbid sample.

4.2.3 Data analysis

The *MS Excel 2010* was used to draw the graphs and carried out one factor ANOVA statistical tool (Garcia et al., 2012).

4.3 Results and discussion

4.3.1 The metals and non-metals in the municipal waste water

Raw municipal waste water sample was submitted to the relevant department for metal and anions analysis using ICP-MS method. The organic and biological composition of waste water is the reflection of the influent water usage. Typical waste water may consist of organic matter which is ± 50 % proteins, 40 % carbohydrates, 10 % fats and oils and trace amounts of priority pollutants and other emerging contaminants (Shon et al., 2007). The concentrations of metals and non-metals of the Malamulele waste water treatment plant collected for this study are presented in Tables 2.

Table 2: Concentrations of metals in municipal waste water sample

Metals and non-metals(ppm)	Al	Fe	Si	Ti	V	Cr	Mn	Co	Li	Be	Mg	Ca	Au	S	Cl
	0.05	20	6.192	<0.05	<0.05	<0.05	0.077	<2	<0.05	<0.05	8.2	28	<0.05	464	27.8

Sulphur was the most abundant non-metal with 464 ppm followed by calcium, chloride and magnesium with 28ppm, 27.8ppm and 8.2ppm respectively.

4.3.2 The pH stability of gold nanoparticles during their transition from light to dark conditions

We investigated the pH stability of citrate functionalised Au-NPs to simulate the discharge and transition of Au-NPs from municipal drinking water flow (under light conditions) into the sewer system (mixed with wastewater under dark conditions). Generally, the increase in pH of Au-NPs suspension to above 7 signifies the decreased concentration of hydrogen ions (H^+) and an increase in hydroxide (OH^-) ions hence the solution becomes alkaline. On the other hand, the decrease in pH to below 7 signifies the increase in hydrogen ions making the solution acidic.

The initial pH of the water samples and gold nanoparticles (Au-NPs) before reconstitution in varying proportions was determined and shown in Figure 1. The pH of the wastewater was slightly alkaline (7.14 ± 0.01), municipal drinking water slightly acidic (6.83 ± 0.02), 20nm Au-NPs was acidic (5.17 ± 0.07) and the 40nm Au-NPs showed very low pH of 3.81 ± 0.01 . Gold nanoparticles used were citrate-stabilized as mentioned above. The 40nm Au-NPs are relatively

unstable as compared to small sized particles and their citrate stabilizers can easily be displaced. The acidic pH of the 40nm gold nanoparticles may be attributed to probably the unstable 40nm Au-NPs forming minimal agglomeration (Au-Au) which leads to displacement of more citrate and may result in residual citric acid and thus low pH (acidic).

The initial pH of the municipal water samples was within the WHO and SABS (SANS 241) guideline value of 5.0 to 9.7 as applicable to South African drinking water quality (WHO, 2011; Ngwenya et al., 2013; DWA, 2013). The initial pH of the municipal waste water was 7.14 ± 0.01 which is within 5.5 to 9.5 ranges as applicable to South African wastewater discharge (DWA, 2009; Odjadjare et al., 2010). The study of He et al. (2007) confirmed that at pH of 7, there were spherical gold nanoparticles of 10 to 20nm size. However on lowering the pH to 4, more triangular gold nanoplates, size 50 to 100nm, appeared in conjunction with spherical gold nanoparticles, size 10 to 50nm.

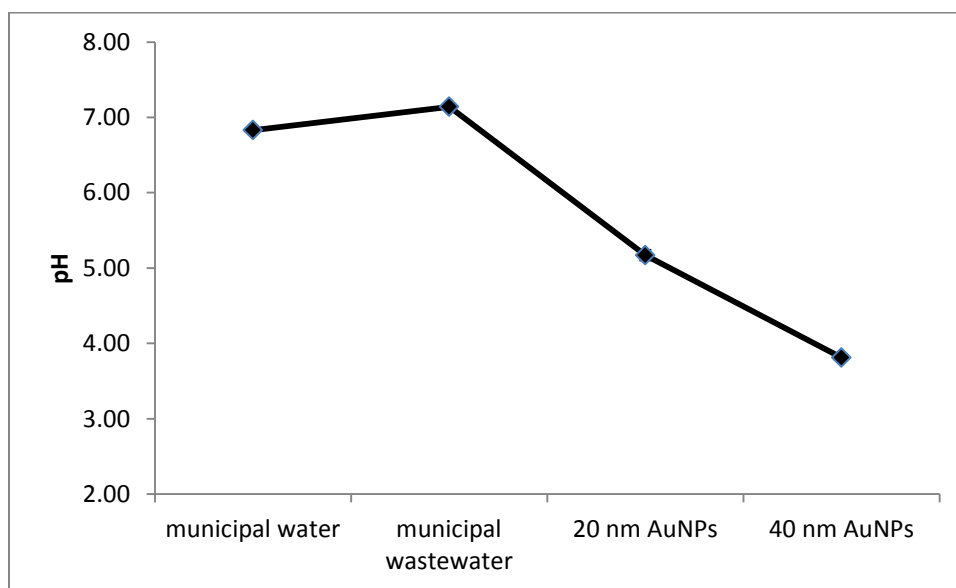


Figure 1: The initial mean pH of the water samples and gold nanoparticles before mixing, whiskers reflect standard deviation of triplicates

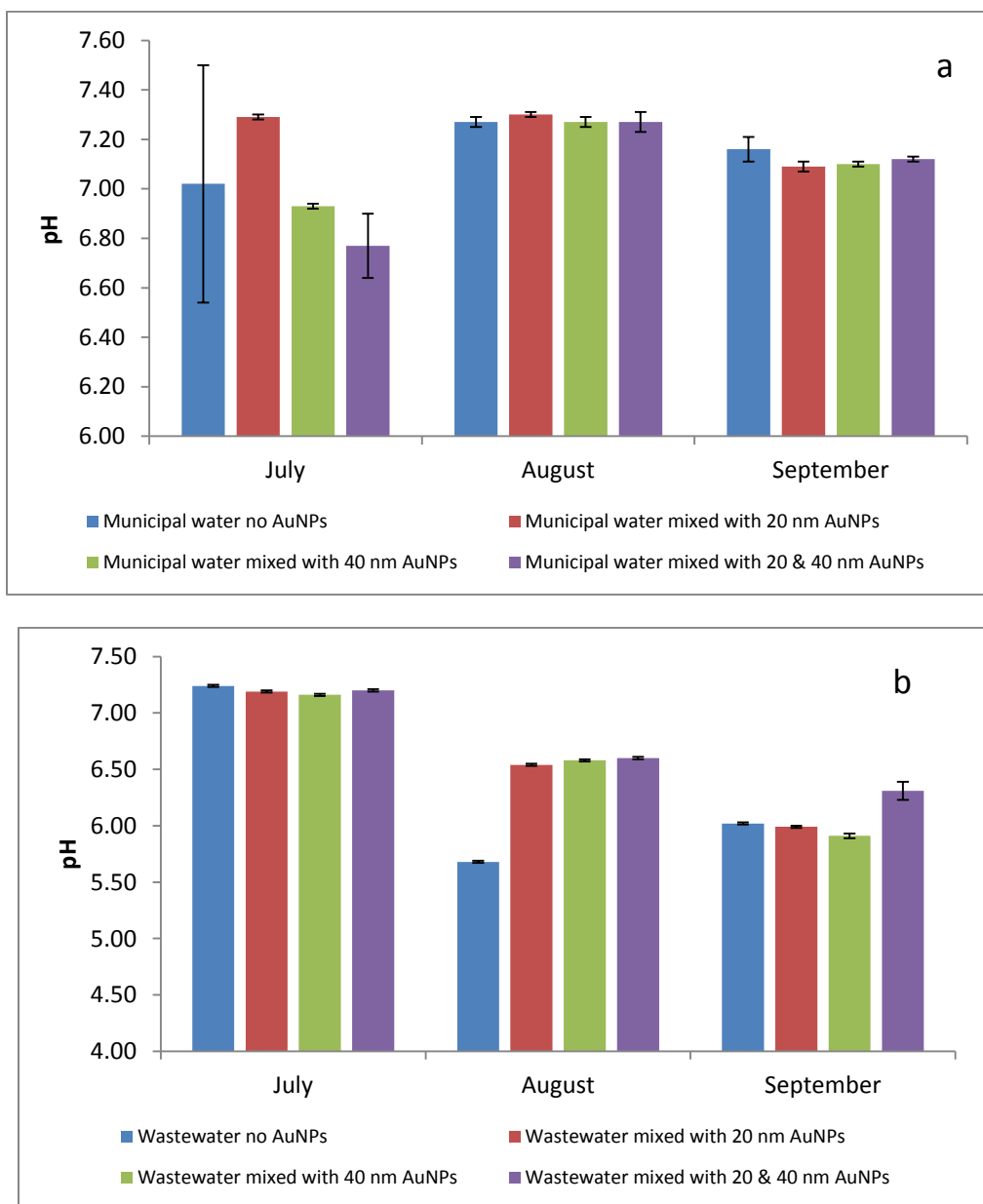


Figure 2: pH variations of (a) municipal water and (b) waste water with and without gold nanoparticles (Au-NPs) in light conditions.

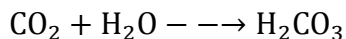
There was a variation in the pH of the municipal water with different mixtures of gold nanoparticles (zero, 20nm, 40nm and mixture of 20nm and 40nm particles) under light conditions (Figure 2a). The municipal water with zero Au-NPs showed a slight change in pH to slightly above 7 in a period of two months and a similar trend of pH in municipal water with Au-NPs was observed (Figure 2a). Thus in two months period, the pH of municipal water with and without Au-NPs was consistently maintained between 6.77 and 7.3. The pH stability of the municipal water may be attributed to the buffering capacity with an alkalinity of 223 mg/l (Van Aardt and Booysen, 2004). The hydrogen carbonate (HCO_3^{2-}) and carbonate (CO_3^{2-}) is responsible for buffering capacity (Owens, 2006). The municipal water with 20nm Au-NPs showed an increase in alkalinity and this can be attributed to the buffering capacity of HCO_3^{2-} and CO_3^{2-} which reacts with acidic 20nm Au-NPs. Thereafter, two months later, through the aging effect the acidic 20nm Au-NPs the pH gradually drops to neutral pH, as indicated by $p=0.00 < 0.05$ which means that there was a significant differences between the data sets. The more acidic 40nm Au-NPs when mixed with municipal water moves to pH 6.93.

Thereafter, two months later, during the aging effect, there was an increase in pH and this can be attributed to the buffering capacity of HCO_3^{2-} and CO_3^{2-} which probably reacts with more acidic 40nm Au-NPs as indicated by $p=0.00 < 0.05$ which means that there was a significant differences between the data sets. The municipal water with a mixture of 20nm and 40nm Au-NPs showed an increase pH from 6.77 to 7.20 in a period of two months and follows a similar pattern as the acidic 20nm and more acidic 40nm Au-NPs.

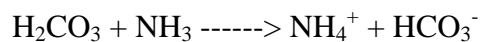
There was a variation in the pH of the municipal wastewater with different mixtures of gold nanoparticles (zero, 20nm, 40nm and mixture of 20nm and 40nm particles) under light conditions (Figure 2b). The municipal waste water (in light environment) with and without gold nanoparticle showed a gradual decrease in pH over a period of two months (Figure 2b). A typical composition of municipal waste water would include microorganisms, organic matter, trace metals, inorganic matter, suspended and dissolved solids amongst others calcium carbonate (CaCO_3) and nitrogen (Pescod, 1992). The decline in pH of municipal waste water with and

without gold nanoparticles may be associated with the presence of carbon dioxide, CaCO_3 and free ammonia in the solution (WHO, 2003).

The carbon dioxide in the atmosphere reacts with water to form carbonic acid



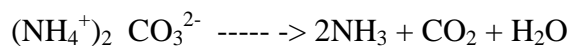
The carbonic acid reacts with a base to the bicarbonate:



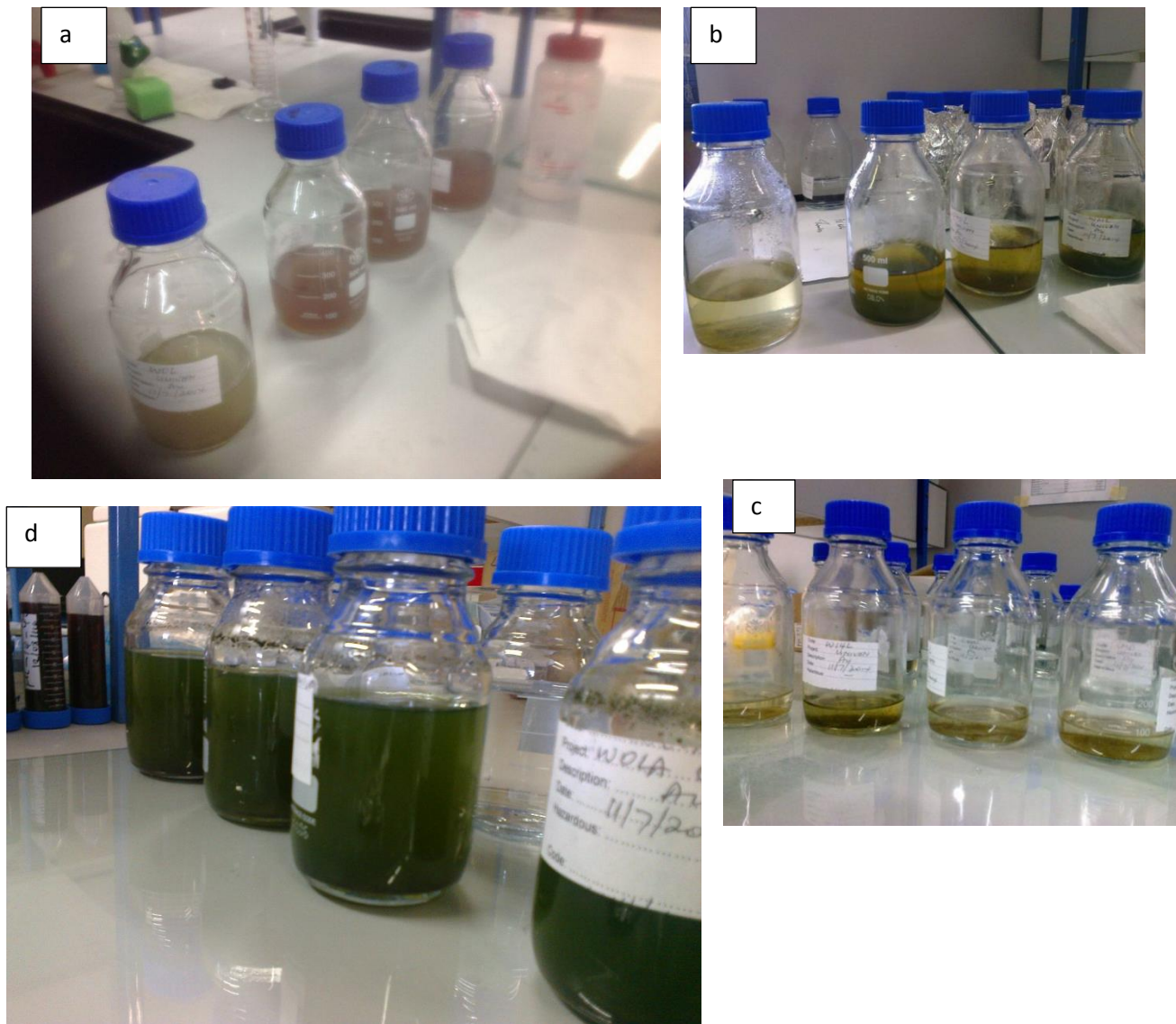
The bicarbonate reacts with ammonia to form ammonium carbonate.



The ammonium carbonate decompose to produce ammonia and carbon dioxide:



The initial acidic gold nanoparticles (20nm at 5.17 and 40nm at 3.81) added in waste water may have also contributed to decline in the pH.



Picture 1:(a)Municipal waste water with and without Au-NPs first day of preparation and were kept under light environment. (b): Municipal waste water with and without Au-NPs kept in light for 1 month. From left to right: waste water with no Au-NPs, waste water with 20nmAu-NPs, waste water with 40nm Au-NPs and waste with a mixture of 20nm and 40nm Au-NPs. (c) municipal waste water with and without Au-NPs in light after 2 months. 1d. municipal waste water with (zero, 20nm, 40nm and a mixture of 20nm and 40nm) Au-NPs after one month under light and agitation condition

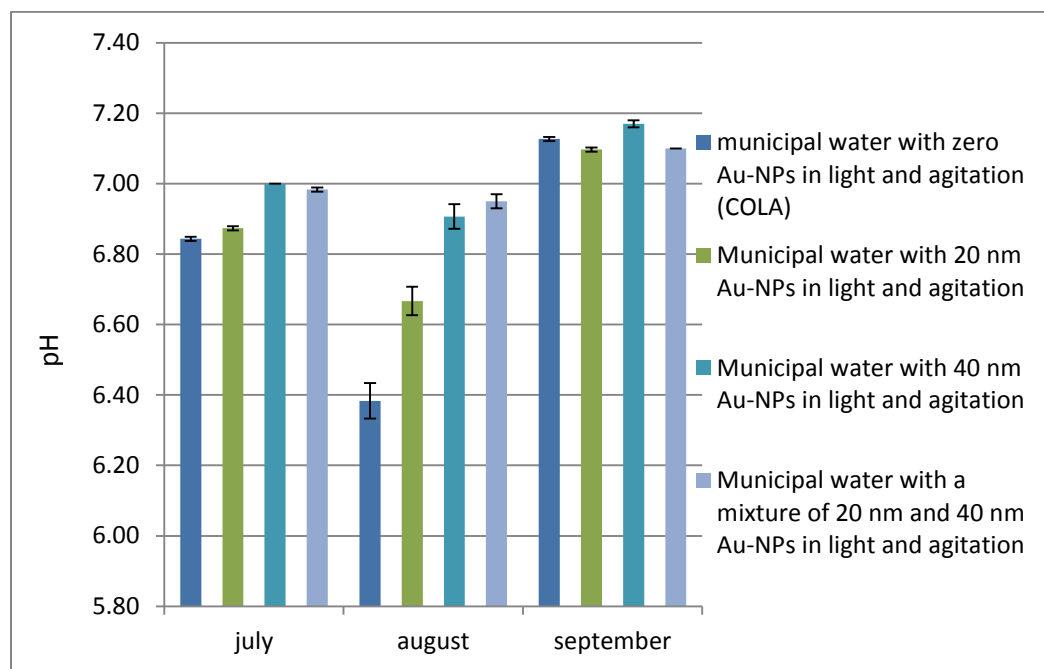


Figure 3: pH variation of municipal drinking water with (zero Au-NPs, 20nm Au-NPs, 40nm Au-NPs, a mixture of 20nm and 40nm Au-NPs) in light and agitation condition.

The $P = 0.019635 < 0.05$ value, showed the significant difference between the data sets (Millar, 2001) from July to September, of the municipal water pH in light and agitation condition. At the onset, just after preparation, the pH of the mixture of Au-NPs and municipal water was nearly the same as the pH of the original water sample (Figure 3). A month later, pH on all the municipal water samples dropped, with municipal water with zero Au-NPs having the highest drop of 6.7% while a mixture of 20nm and 40nm having the lowest decline in pH of 0.4%. The error bars suggest that there could be a possible error on the August data as shown on the Figure 3. On the second month (September), all the pH increased to just above pH 7 suggesting that hydrogen carbonate (HCO_3^-) and carbonate (CO_3^{2-}) (from carbonic acid in water) may be responsible for the increase in pH (Owens, 2006).

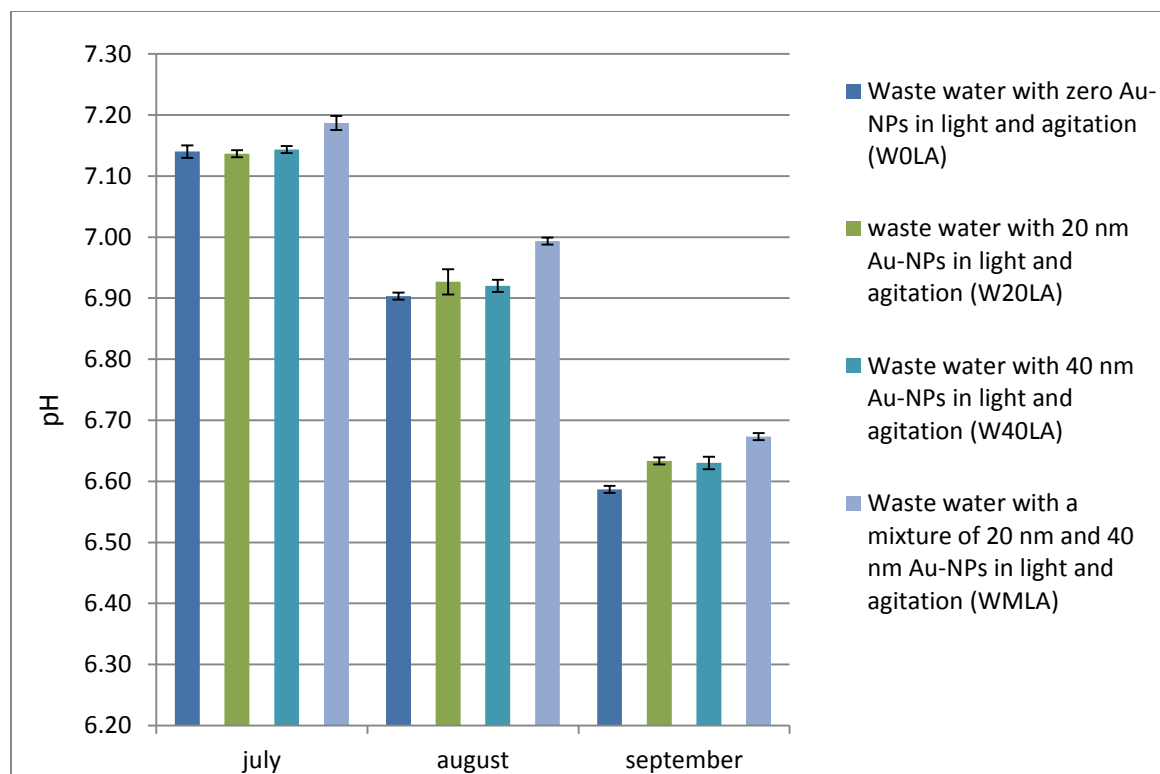


Figure 4 : pH variation of municipal waste water with (zero Au-NPs, 20nm Au-NPs, 40nm Au-NPs, a mixture of 20nm and 40nm Au-NPs) in light and agitation condition.

The data on the Figure 4 presents the declining pH over the period of two months. There was a significant difference in the data sets as indicated by $P = 0.00 < 0.05$. As the waste water was aging, the colour changed to green (Picture 1d). The green colour has been probably caused by the growth of algae. Even though algae are known to consume carbon dioxide, the possible higher quantities of ammonia react with bicarbonates to produce more carbon dioxide. The drop in pH of waste water (with and without gold nanoparticles) may probably be associated with an increase in carbon dioxide concentration in the solution (WHO, 2003). There was no effect caused by the presence of Au-NPs in the waste water.

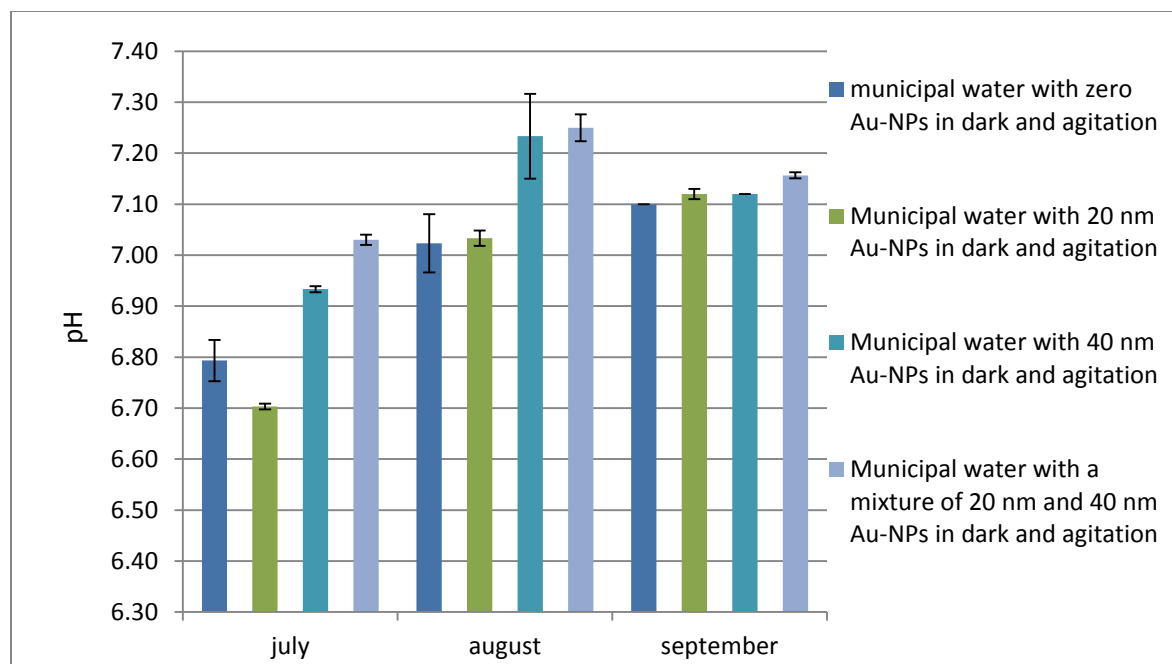


Figure 5: pH variation of municipal drinking water with (zero Au-NPs, 20nm Au-NPs, 40nm Au-NPs, a mixture of 20nm and 40nm Au-NPs) in dark and agitation condition.

There was an increase in pH in August (during the first month of incubation) on municipal drinking water with zero Au-NPs, 20nm Au-NPs, 40nm Au-NPs and a mixture of 20nm and 40nm Au-NPs (Figure 5). Municipal drinking water with 40nm Au-NPs and a mixture of 20nm Au-NPs and 40nm Au-NPs showed the highest increase in pH to above 7.2 in August. There are organisms and species that grow in water including algae that can consume carbon dioxide from the system (carbon fixation) (Fitch and Kemker, 2014). The reduction in CO₂ levels lead to the increase in pH (Kemker, 2013). In the second month of the study, there was a slight increase in pH on the municipal water with zero Au-NPs and 20nm Au-NPs while the 40nm Au-NPs and mixture of 20nm and 40nm Au-NPs decreasing down to slightly above 7 (Figure 5). Excluding the municipal drinking water with 40nm Au-NPs and a mixture of 20nm Au-NPs and 40nm Au-NPs in August because of their erratic sign on error bars, the trend of the pH on the last two months (August and September) showed a consistent pH at just above 7. This consistency of pH to just above neutral can be attributed to the buffering system by hydrogen carbonate (HCO₃⁻) and carbonate (CO₃²⁻) to maintain pH level (Owens, 2006). There was no effect of the presence of Au-NPs on pH observed in the municipal water

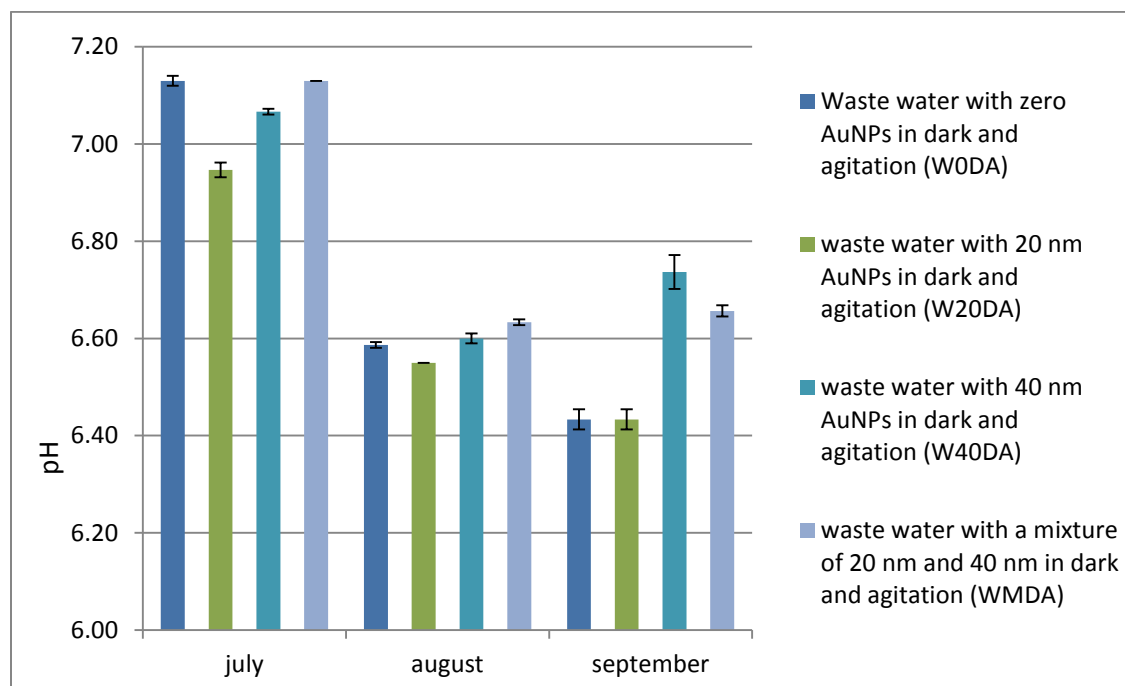


Figure 6: pH variation of municipal waste water with (zero Au-NPs, 20nm Au-NPs, 40nm Au-NPs, a mixture of 20nm and 40nm Au-NPs) in dark and agitation condition.

There was a decrease in pH on waste water with (zero Au-NPs, 20nm Au-NPs, 40nm Au-NPs, a mixture of 20nm and 40nm Au-NPs) in the first month of incubation (Figure 6). There was a further decline in pH of waste water with zero Au-NPs and waste water with 20nm Au-NPs while there was a slight increase on waste water with 40nm Au-NPs and a mixture of 20nm and 40nm Au-NPs. The increase in pH on the 40nm Au-NPs may probably be the invalidity of data as shown by error bar on the figure (Millar, 2001). Carbon dioxide is the most common cause of acidity in water (Kemker, 2013).

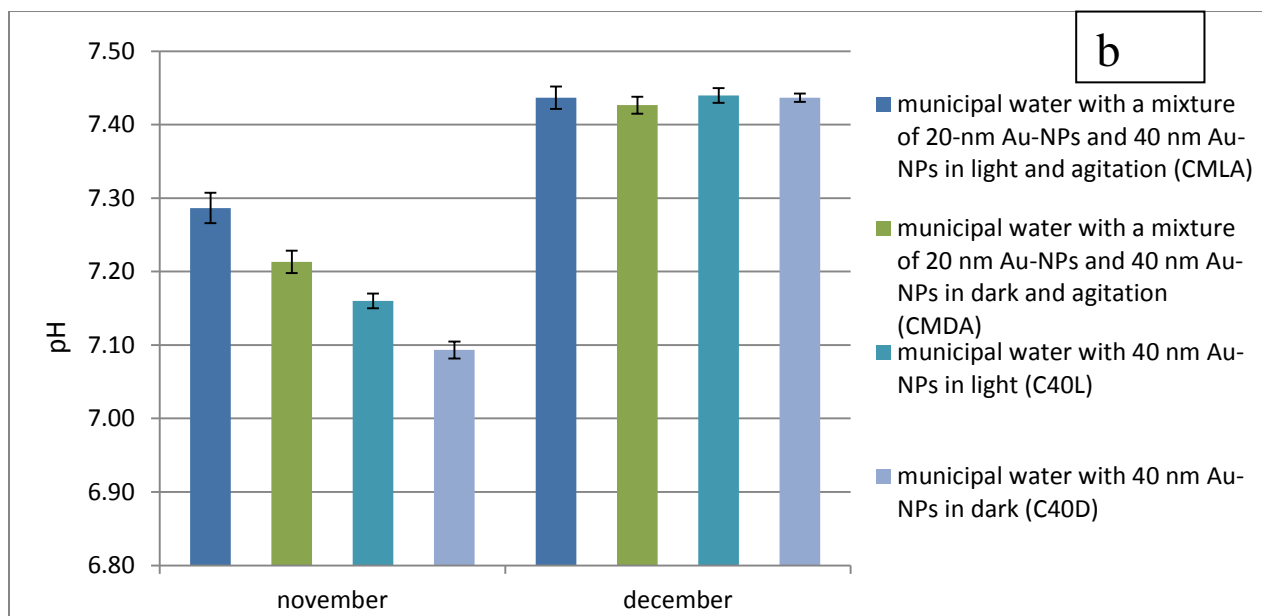
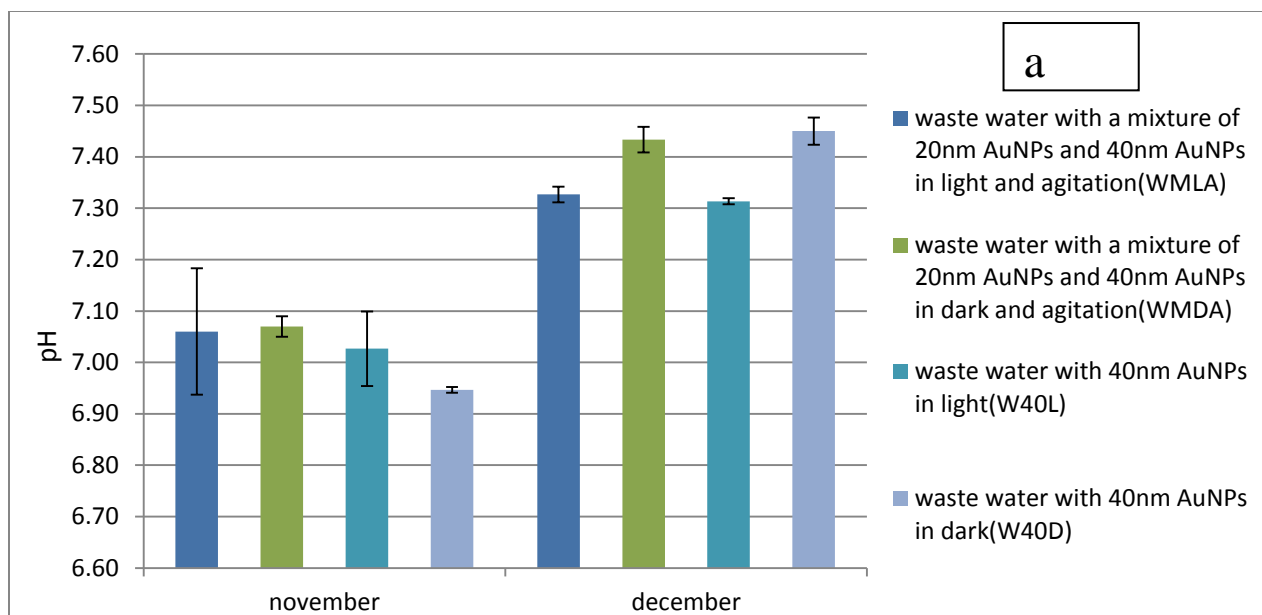


Figure 7: pH variations under anaerobic conditions of (a) municipal waste water with a mixture of 20nm and 40nm Au-NPs in light and agitation, a mixture of 20nm and 40nm Au-NPs in dark and agitation, 40nm Au-NPs in light and lastly 40nm Au-NPs in dark (b) municipal water with a mixture of 20nm and 40nm Au-NPs in light and agitation, a mixture of 20nm and 40nm Au-NPs in dark and agitation, 40nm Au-NPs in light and lastly 40nm Au-NPs in dark.

Under anaerobic conditions, both municipal drinking water and municipal waste water with and without Au-NPs had an increase in pH in two months. The pH increased to the more alkaline levels (>7) (Figure 7).

4.3.3 The interaction of gold nanoparticles and organic matter (chemical oxygen demanding substances) during their transition from light to dark conditions

The municipal drinking water sources contain natural organic matter (NOM). Natural organic matter is a key component in aquatic environment and is also present in fresh water sources. NOM are amorphous, dark coloured and acidic in nature and is composed either of low molecular weight ($<3\text{kDa}$) hydrophobic aliphatic ketones and alcohols or large molecular weight hydrophobic (>3 to 500kDa) compounds such as phenolic groups, aromatic rings and conjugated bonds (Chowdhury, 2013). NOM influences acidity, mobility and toxicity of metals and organic pollutants (Sillanpaa, 2014). The municipal waste water (sewage) is rich in organic compounds with over 300 being identified so far (Wilson et al., 1996). The waste water is also rich in nutrients (nitrogen, phosphorus) which are essential for bacterial growth (Shon et al., 2007).

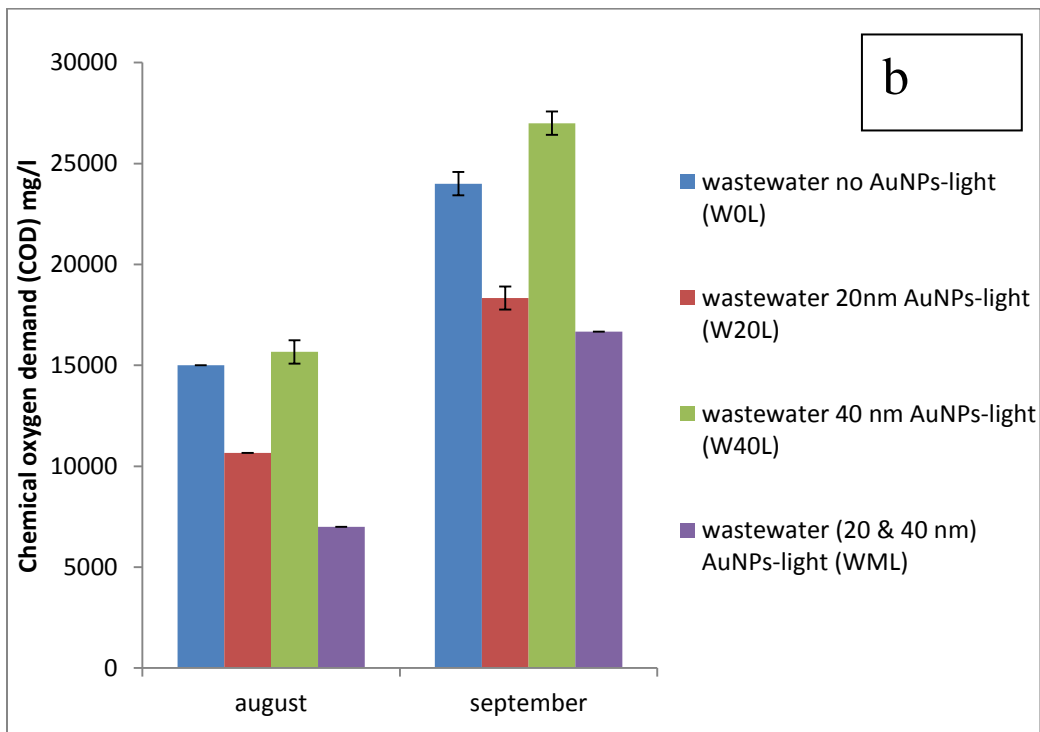
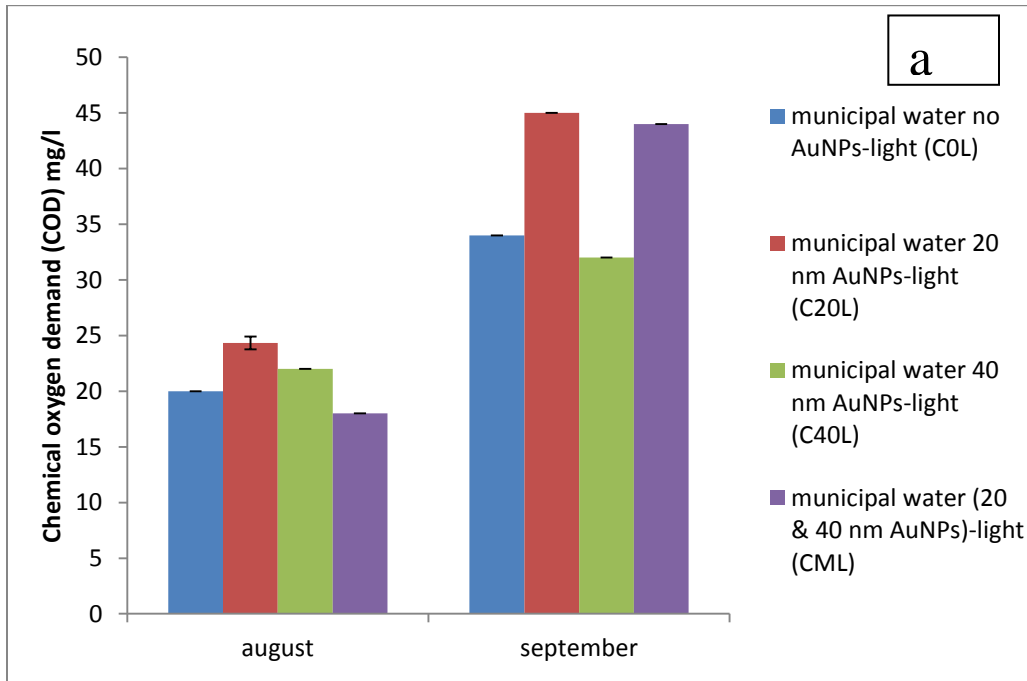


Figure 8: Chemical oxygen demand (COD) of (a) municipal water and (b) wastewater with (zero, 20nm Au-NPs, 40nm Au-NPs and a mixture of 20nm and 40nm Au-NPs) in light condition, no agitation

Under aerobic conditions the municipal water (Figure 8a) and waste water (Figure 8b) with and without Au-NPs, showed an increase in the levels of chemical oxygen demand (COD) in two months. There was a significant difference between the two months data set, hence $p = 0.002747 < 0.05$ and $p = 0.024344 < 0.05$ for municipal water and waste water respectively. Municipal drinking water sources contain natural organic matter (Chowdhury, 2013; Sillanpaa, 2014). The increase in COD in September shows some possible accumulation of organic matter which might be attributed to the presence of natural organic matter. Natural organic matter is present in fresh water and obviously ends up in waste water. Waste water is composed of, but not limited to, pesticides, volatile organic matter, chloride, sulphur heavy metals among other constituents (Shon et al., 2007). Natural organic matter (NOM) and/or other inorganic substances in water react with chlorine and other disinfectants to produce disinfection by-products (DBPs) in water (Chowdhury, 2013). Thus natural organic matter and the capping agent citrate (organic acid) (Glenn et al., 2012) of the gold nanoparticles (Au-NPs), contribute to the accumulation of organic matter which leads to the increases in the COD levels.

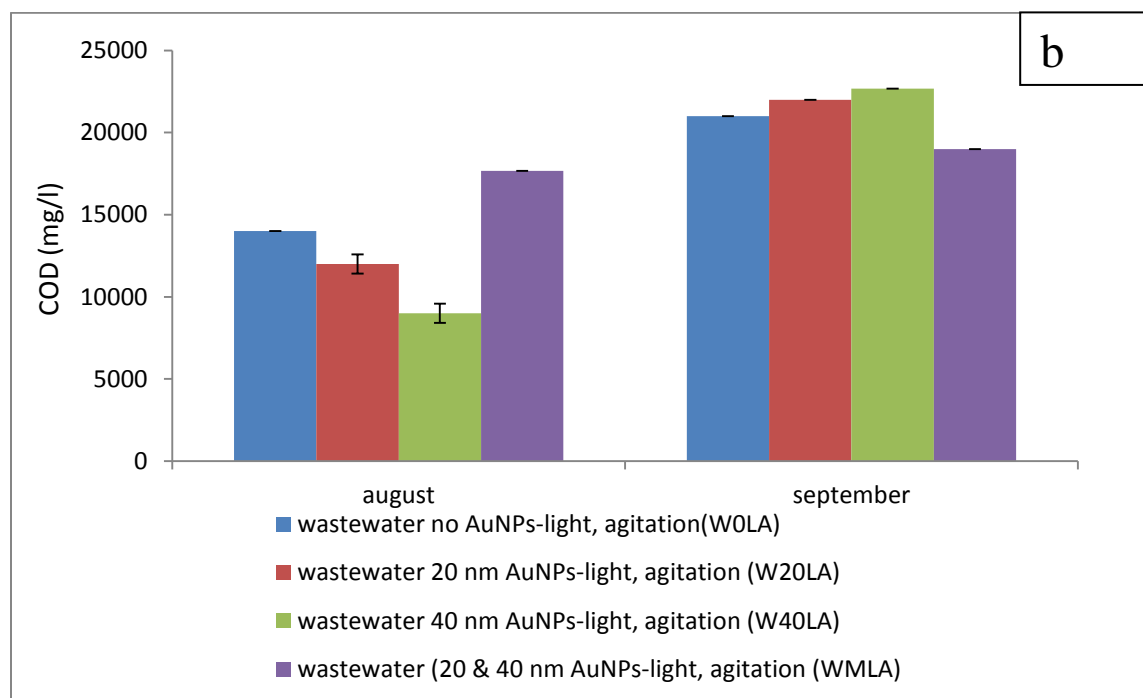
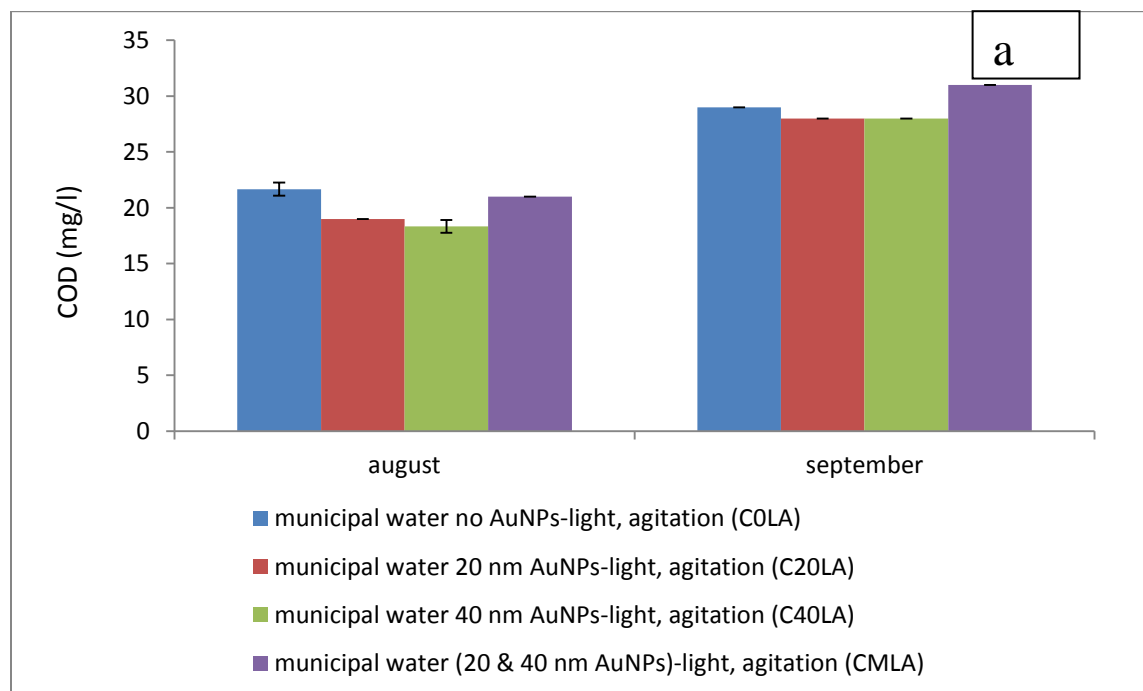


Figure 9: Chemical oxygen demand (COD) of (a) municipal water and (b) waste water with (zero, 20nm Au-NPs, 40nm Au-NPs and a mixture of 20nm and 40nm Au-NPs) in light and agitation condition.

In light and agitation, both municipal water (Figure 9a) and waste water (Figure 9b) also showed an increase in COD in the two months period. Thus agitation did not contribute to any alteration of the COD trend under light with no agitation as in Figure 9.

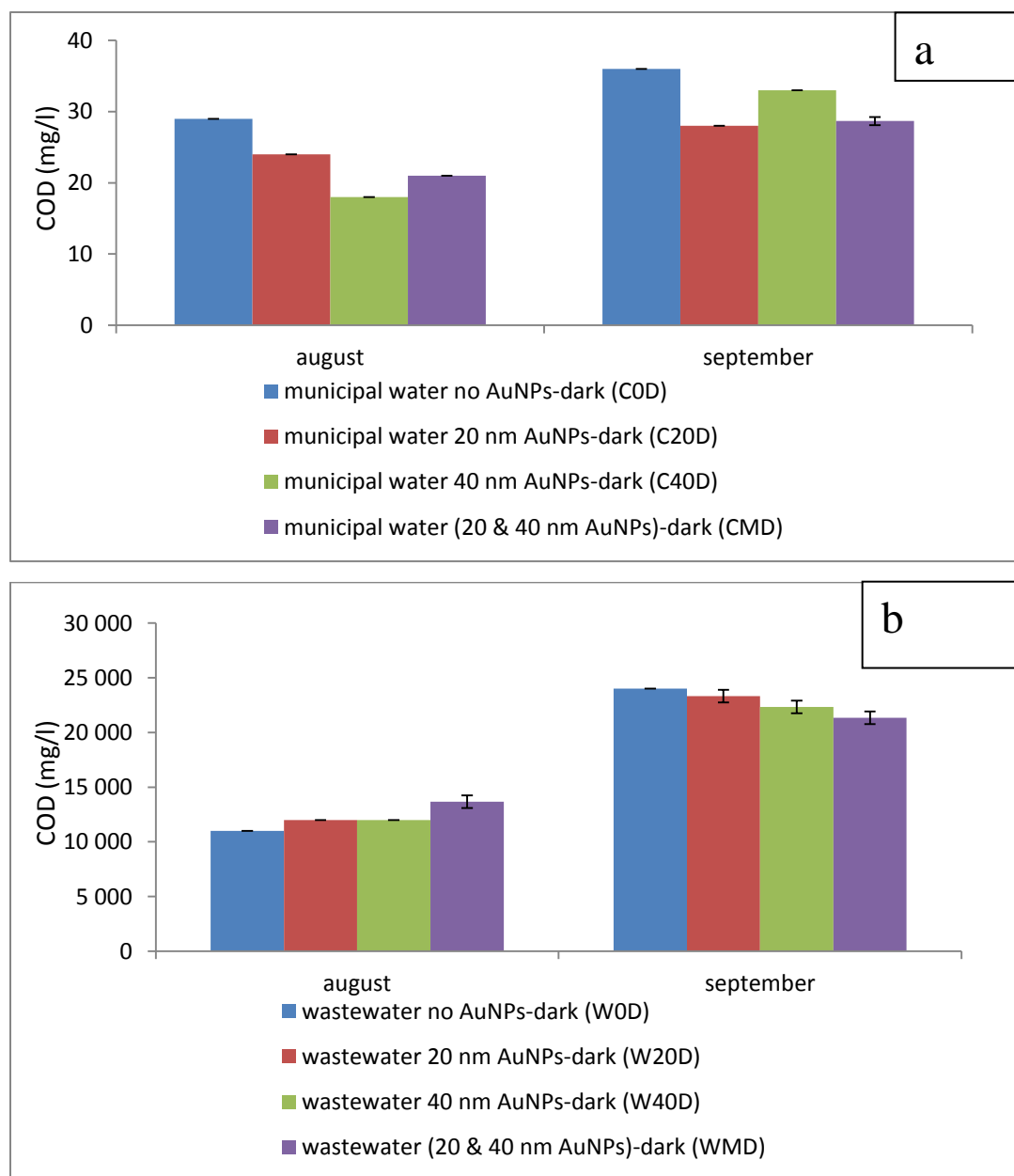


Figure 10: Chemical oxygen demand (COD) of (a) municipal water and (b) waste water with (zero, 20nm Au-NPs, 40nm Au-NPs and a mixture of 20nm and 40nm Au-NPs) in dark (away from direct light) condition

The municipal water and waste water with and without Au-NPs in the dark (away from light) showed an increase in COD in a two months period (Figure 10). The absence / scarcity of light did not make any impact on the reaction as they share the same trend with samples kept in light and was similar to Figures 8 and 9.

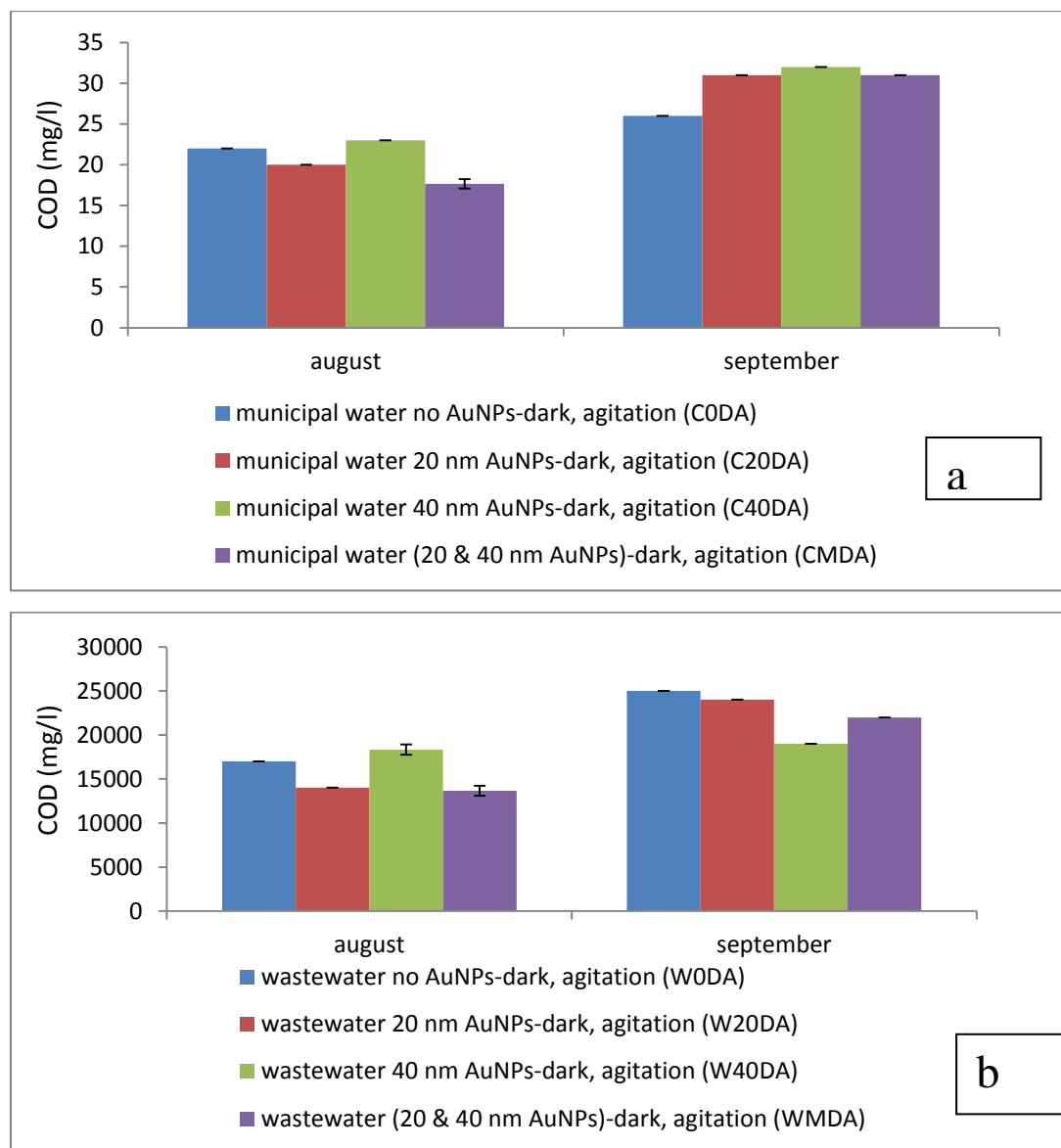


Figure 11: Chemical oxygen demand (COD) of municipal water (a) and waste water (b) with (zero, 20nm Au-NPs, 40nm Au-NPs and a mixture of 20nm and 40nm Au-NPs) in dark and agitation condition

Both the municipal water and waste water in dark and agitation (Figure 11) showed a general increase in COD as is the case in dark without agitation. Light scarcity and agitation did not change the trend of increasing COD in two months period and was similar to Figures 8 to 10.

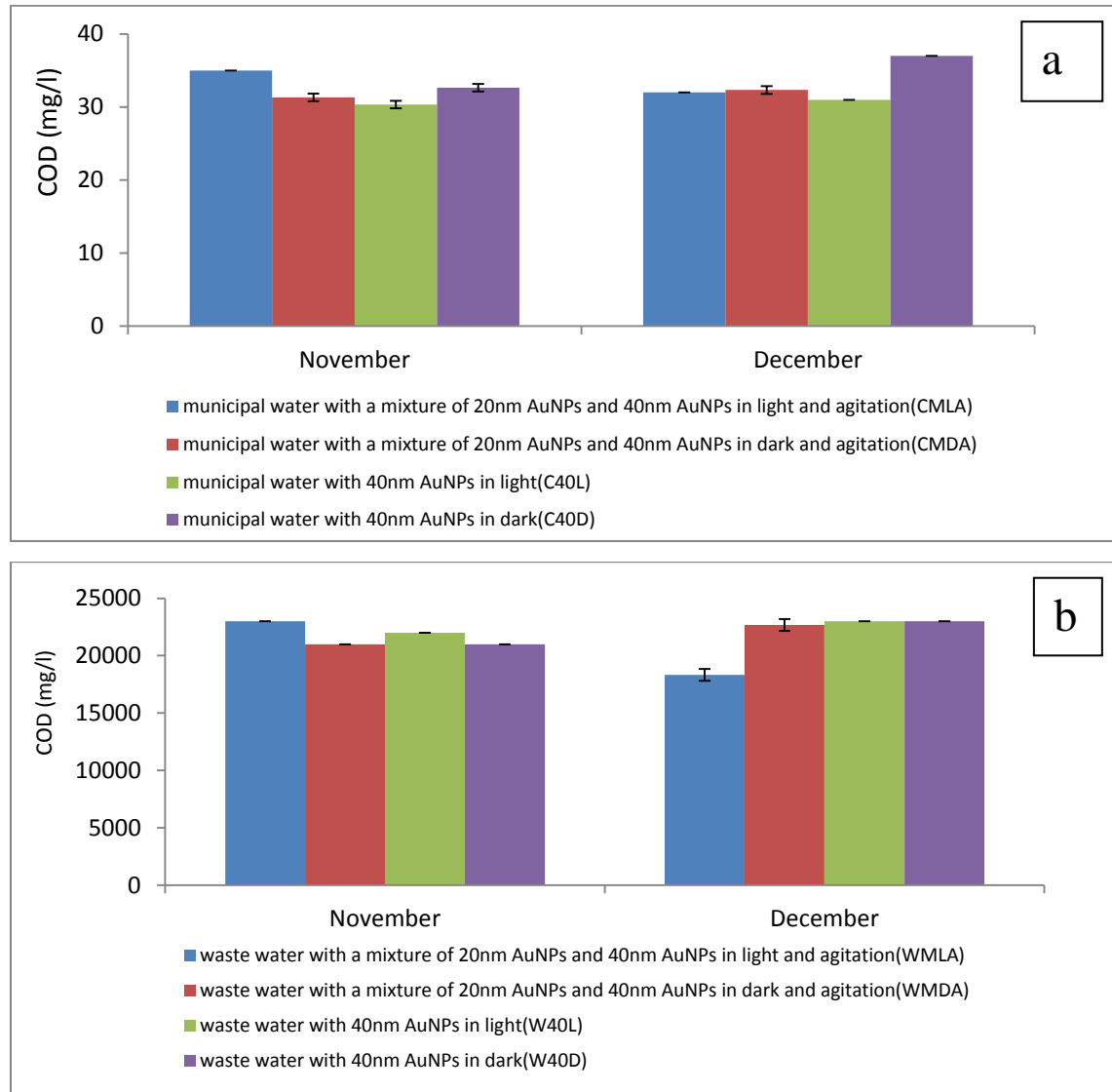


Figure 12: (a) Municipal water with a mixture of 20nm and 40nm Au-NPs under light and agitation and dark and agitation and water with 40nm Au-NPs in light and also in dark all under anaerobic condition. (b) COD variations of waste water with a mixture of 20 and 40nm Au-NPs in light/dark with agitation and waste water with 40nm Au-NPs in light/dark all under anaerobic condition.

Under anaerobic conditions, the COD of municipal water and wastewater (Figure 12) in two months period remained at almost constant levels. There was a slight decrease in COD of waste water with a mixture of 20 and 40nm Au-NPs in light and agitation. Waste water with a mixture of 20nm and 40nm Au-NPs in dark and agitation, waste water with 40nm Au-NPs in light and waste water with 40nm Au-NPs in dark, all presented a very slight increase in COD. These can be assumed as the constant levels (no significant change) of COD over the two months period.

Comparing the COD variations in municipal water with Au-NPs under aerobic and anaerobic condition, there was an overall increase in COD under aerobic condition and no significant change in COD was established under anaerobic condition. These suggest that there might probably be microorganisms that produce inorganic nanostructure and metallic nanoparticles (Gericke and Pinches, 2006) probably only under aerobic condition. As mentioned earlier, natural organic matter is found in all fresh water and absolutely end up into waste water after use. Anaerobic condition has the low energy requirement and can be used to convert organic matter into methane and carbon dioxide (de Mes et al., 2003). Different groups of microorganisms are capable of different anaerobic processes in the course of biodegrading organic matter. Non-soluble biopolymers are converted to soluble organic compounds during hydrolysis and the process is catalysed by enzymes excreted by acidogenic bacteria and convert soluble organic compounds to volatile fatty acids and alcohols. Volatile fatty acids and alcohols are converted to acetate and hydrogen (H_2) in acetogenesis which in the end acetate and carbon dioxide and hydrogen produce methane gas conducted by methanogenic bacteria (de Mes et al., 2003). Some of these nutrients are essential for bacterial growth. The waste water used (from Malamulele waste water plant) had a very high level of COD (34667 mg/l) which makes it (waste water) eutrophic and supports the growth of most heterotrophic bacteria (Sillanpaa, 2015) algae (algal bloom) and other aquatic plants. Generally, waste water is composed of carbohydrates, fats, oils, grease, volatile organic matter, heavy metals, phosphorus, sulphur, hydrogen sulphide, nitrogen, chlorides and oxygen (Shon et al., 2007).

Some of these microorganisms' breaks down to organic matter contributing to the natural organic matter (NOM) thus probably increasing the COD levels significantly in the waste water

(Stankus et al., 2010). Algal growth in the waste water was demonstrated by the green colour of the waste water solutions with and without Au-NPs. As oxygen is one of the most important nutrient for some bacterial growth, its depletion automatically limits the growth of these microorganisms and thus limits the proliferation and breakdown to organic matter hence no significant increase in COD under anaerobic condition. Gold nanoparticles (Au-NPs) addition to waste water did not show any significant effect on the COD of the waste water but oxygen availability had an effect on the COD levels.

4.4 Conclusion

The metal and non-metals analysis of raw municipal waste water revealed the abundants of sulphur, calcium, chlorides, magnesium, silicon and some trace metals. The study confirmed the absence of Au⁰ in the raw waste water used. The municipal waste water (in light environment) with and without gold nanoparticle showed a gradual decrease in pH over a period of two months under aerobic conditions. Under anaerobic conditions, both municipal drinking water and municipal waste water with and without Au-NPs had an increase in pH in two months. The pH increased to the alkaline levels. Gold nanoparticle did not show any impact on the pH change in the waste water as the water without Au-NPs were following the same trend as the samples loaded with Au-NPs. Comparing the COD variations in municipal water with Au-NPs under aerobic and anaerobic condition, there was an overall increase in COD under aerobic condition and no significant change in COD was established under anaerobic condition. These suggest that there might probably be microorganisms that produce inorganic nanostructure and metallic nanoparticles. All municipal water and waste water with and without Au-NPs under aerobic condition showed a general increase in COD in a period of two months while the same type of samples kept under anaerobic condition did not show a significant change in COD over the same period. The presence of Au-NPs in the waste water did not show any possible influence to the COD variation.

Chapter 5: The Hydrodynamic diameter of Gold nanoparticle (Au-NPs) in the different water environments

Abstract

The zeta potential was used to determine the stability of nanoparticle in water. The stability of gold nanoparticles in municipal drinking water and waste water was investigated. The study showed that stable citrate functionalised Au-NPs disposed into the water were destabilized after making contact with water both under aerobic and anaerobic conditions. The zeta potential of Au-NPs in waste water in light and agitation showed the stable particles in the first month and the unstable particles the second month. This study showed that Au-NPs released into waste water might end up losing their stability and thus affect their reactivity.

5.1 Introduction

This Dynamic light scattering (DLS) technique determines the hydrodynamic diameter of colloidal particles which include the electrostatic double layer around particles (Hotze et al., 2010). It is used to study the distribution of particles and the extent of agglomeration of particles that occurs during the experiment (Garcia et al., 2012). DLS determine the hydrodynamic diameter of a particle (in d.nm) while TEM micrographs gives a true radius of a particle (Lim et al., 2013). Zeta potential measurements allow the study of the surface properties and any change in the surface of particles. This technique studies the stability of nanoparticles and their surface charge when they are electrostatically stabilized (Garcia et al., 2012).

5.2 Materials and Methods

5.2.1 Zeta potential (Zp)

The cuvettes were carefully filled with the sample to avoid formation of bubbles in the cuvette. Filled cuvettes were put in the cuvette slot of the zetalyzer Nano ZS systems (Malvern instruments, 2012) machine, operating at light source wavelength of 532 nm and a fixed scattering angle of 173° for detection. The sample was pre-warmed for 120 minutes before running the test. The results were recorded in triplicates.

5.2.2 Data analysis

The *MS Excel 2010* was used to draw the graphs and carried out one factor ANOVA statistical tool (Garcia et al., 2012).

5.3 Results and discussion

The gold nanoparticles (Au-NPs) in municipal drinking water and waste water exhibited negative variation in zeta potentials (Figures 13 to 17). The zeta potential indicates the stability of gold nanoparticles thereby predicting the interaction between nanoparticles and biological cells (Zhang et al., 2008). The municipal drinking water under aerobic and light conditions (no agitation) had Au-NPs charged between -14 mV and -18 mV for a period of two months (Figure 13a). The waste water with 20 nm Au-NPs and waste water with a mixture of 20 nm and 40 nm Au-NPs in aerobic and light conditions (no agitation) exhibited zeta potential of -29.1 mV and -27.8 mV respectively on the first month (Figure 13b). On the second month, their charges were decreased in magnitude to -19.7 mV and -20.8 mV (Figure 13b). Waste water with zero and 40 nm Au-NPs maintained the zeta charge of around -20 mV for the period of two months. The decrease in zeta potential (positive side) showed the loss of stability of nanoparticles.

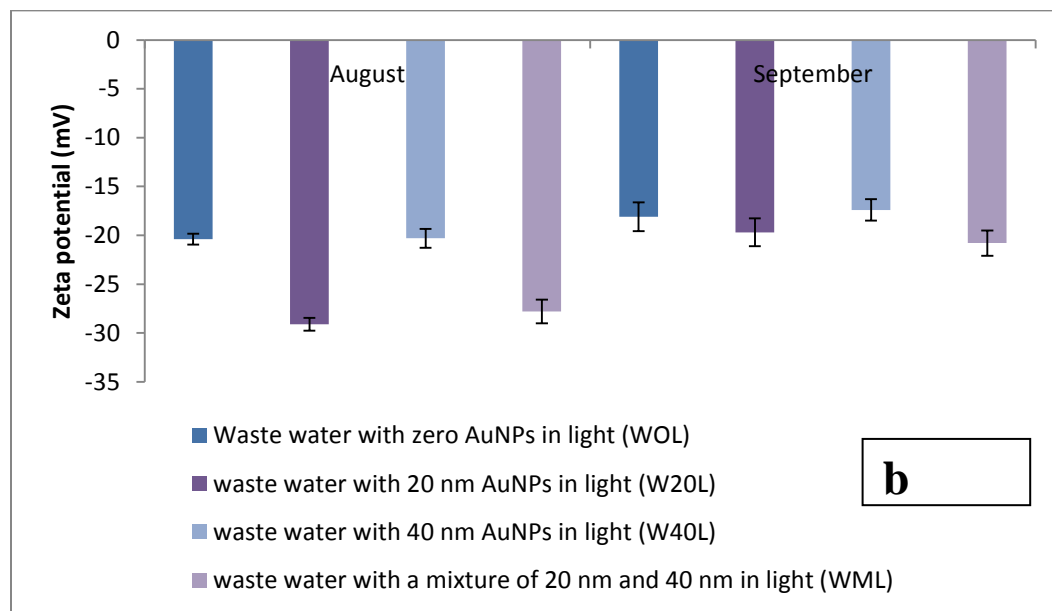
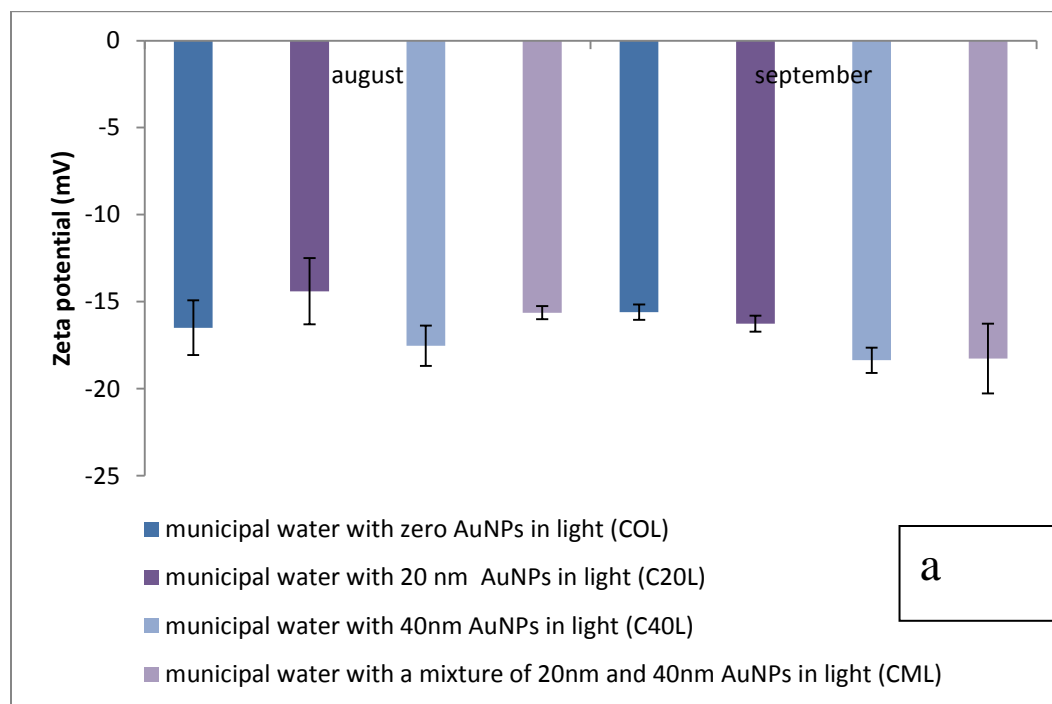


Figure 13: (a) Zeta potential of municipal water with zero, 20nm, 40nm and a mixture of 20nm and 40nm Au-NPs. (b) Zeta potential of waste water with zero, 20nm, 40nm and a mixture of 20nm and 40nm Au-NPs under aerobic and light conditions (no agitation) for two months period.

Waste water with 20nm Au-NPs and waste water with a mixture of 20nm and 40nm Au-NPs In light showed the zeta potential -29.1mV and -27.8mV respectively in the first month after mixing

(Figure 13 b). This particles stability was probably transferred from the original 20nm charge of -31 mV. That stability was lost in the second month and the charge was decreased in magnitude to about -20mV.

The municipal drinking water with Au-NPs had zeta potential at a range between -12.6 mV and -18.2mV under aerobic and light conditions with agitation (Figure 14a). The waste water with zero, 20nm, 40nm and a mixture of 20nm and 40nm Au-NPs under aerobic and light conditions with agitation had zeta potential of almost -30mV in August (Figure 14b). Later in September, all zeta potential decreased in magnitude to below -20mV. The significant loss of stability of gold particles on the second month of incubation might be attributed to agitation. Agitation increases the oxygen and other waste water components bioavailability and presumably promotes the displacement of the capping agent (citrate in this case) as it (citrate) is weakly bound and easily displaced (nanocomposix.com).

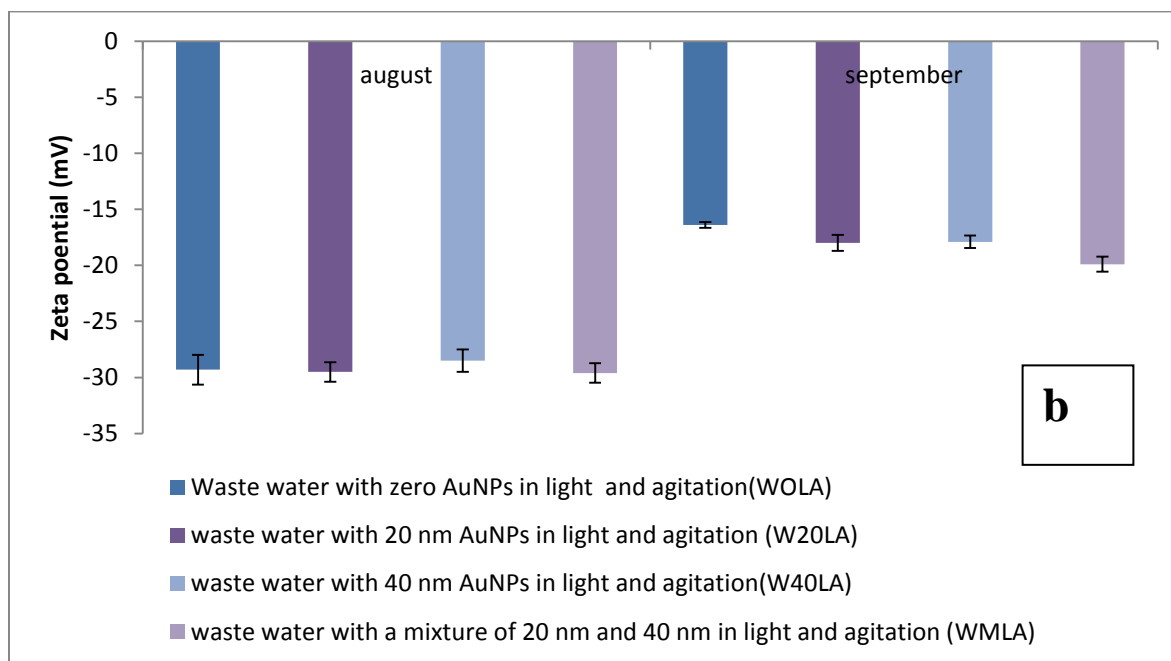
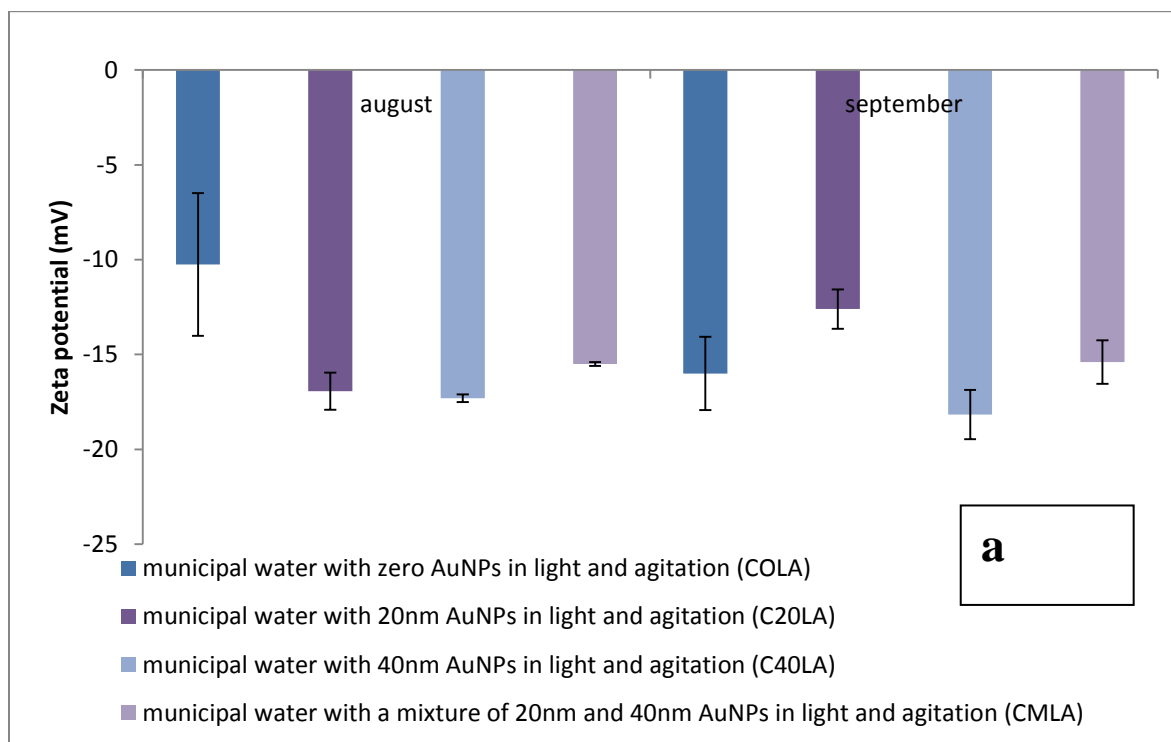


Figure 14: (a) Zeta potential from municipal water with zero, 20nm, 40nm and a mixture of 20nm and 40nm Au-NPs. (b) Zeta potential from waste water with zero, 20nm, 40nm and a mixture of 20nm and 40nm Au-NPs under aerobic and light conditions with agitation for two months period.

Then under aerobic and dark conditions (no agitation) the Au-NPs in the municipal water maintained the zeta charge that ranged between -14.3 mV and -17.3 mV (Figure 15a). The Au-NPs in waste water with and without Au-NPs in aerobic and dark conditions showed a slight decrease in the zeta charge (Figure 15b).

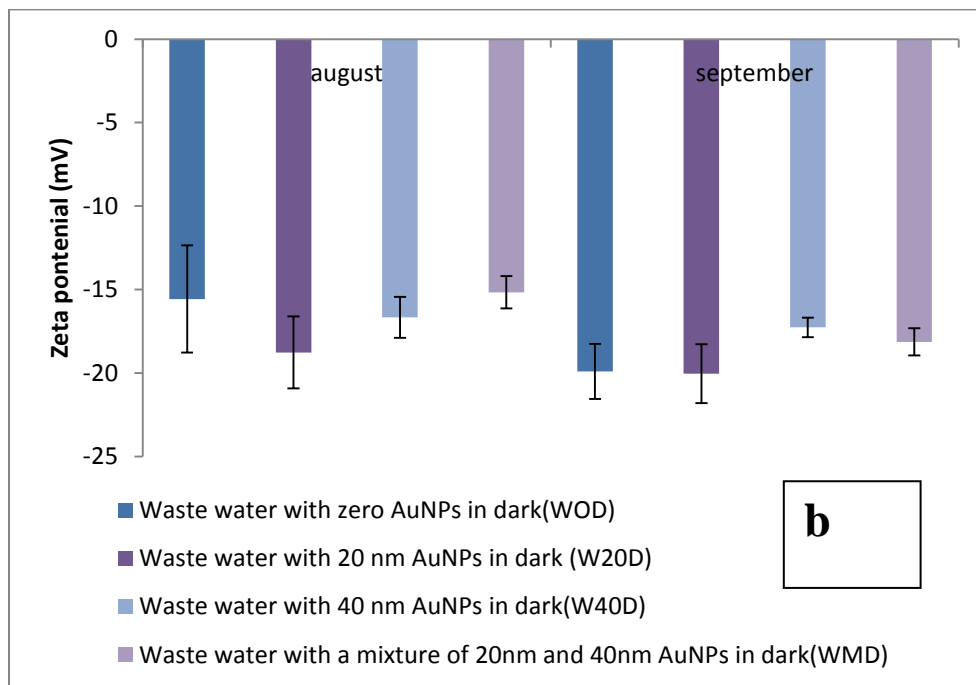
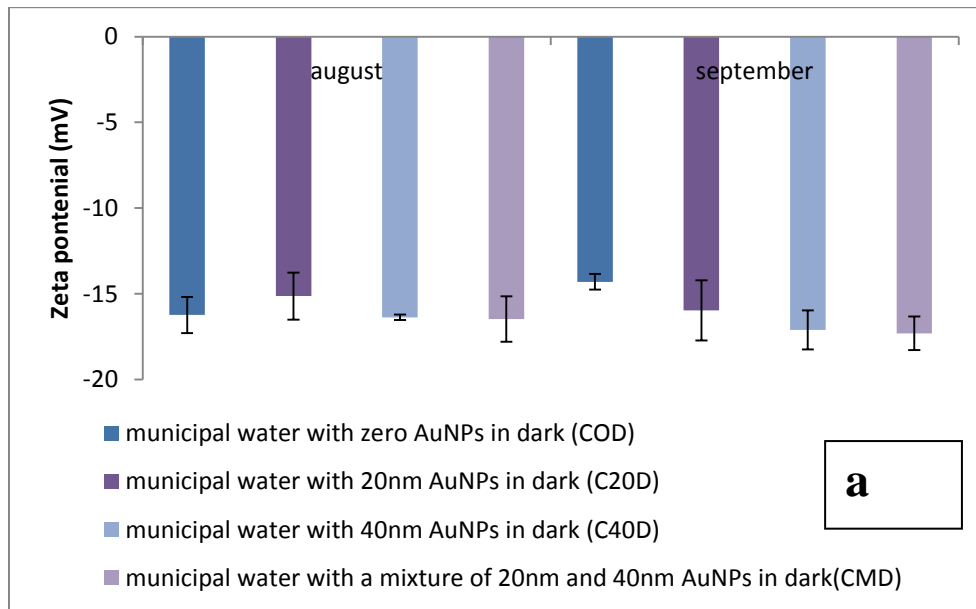


Figure 15: (a) Zeta potential from municipal water with zero, 20nm, 40nm and a mixture of 20nm and 40nm Au-NPs. (b) Zeta potential from waste water with zero, 20nm, 40nm and a mixture of 20nm and 40nm Au-NPs under aerobic and dark conditions (no agitation) for two months period.

Then under aerobic and dark conditions with agitation municipal drinking water with and without Au-NPs presented almost consistent zeta potentials ranging between -13.9 mV and -18.7 mV (Figure 16a). The Au-NPs in waste water with zero and 20nm Au-NPs had a slight increase in charge from -19.8mV and -20.1mV to -16.4mV and -18.1mV respectively (Figure 16b). The waste water with 40nm Au-NPs maintained its charge at -18mV while waste waters with a mixture of 20nm and 40nm Au-NPs exhibits a decrease in charge from -19.6mV to -23.4mV.

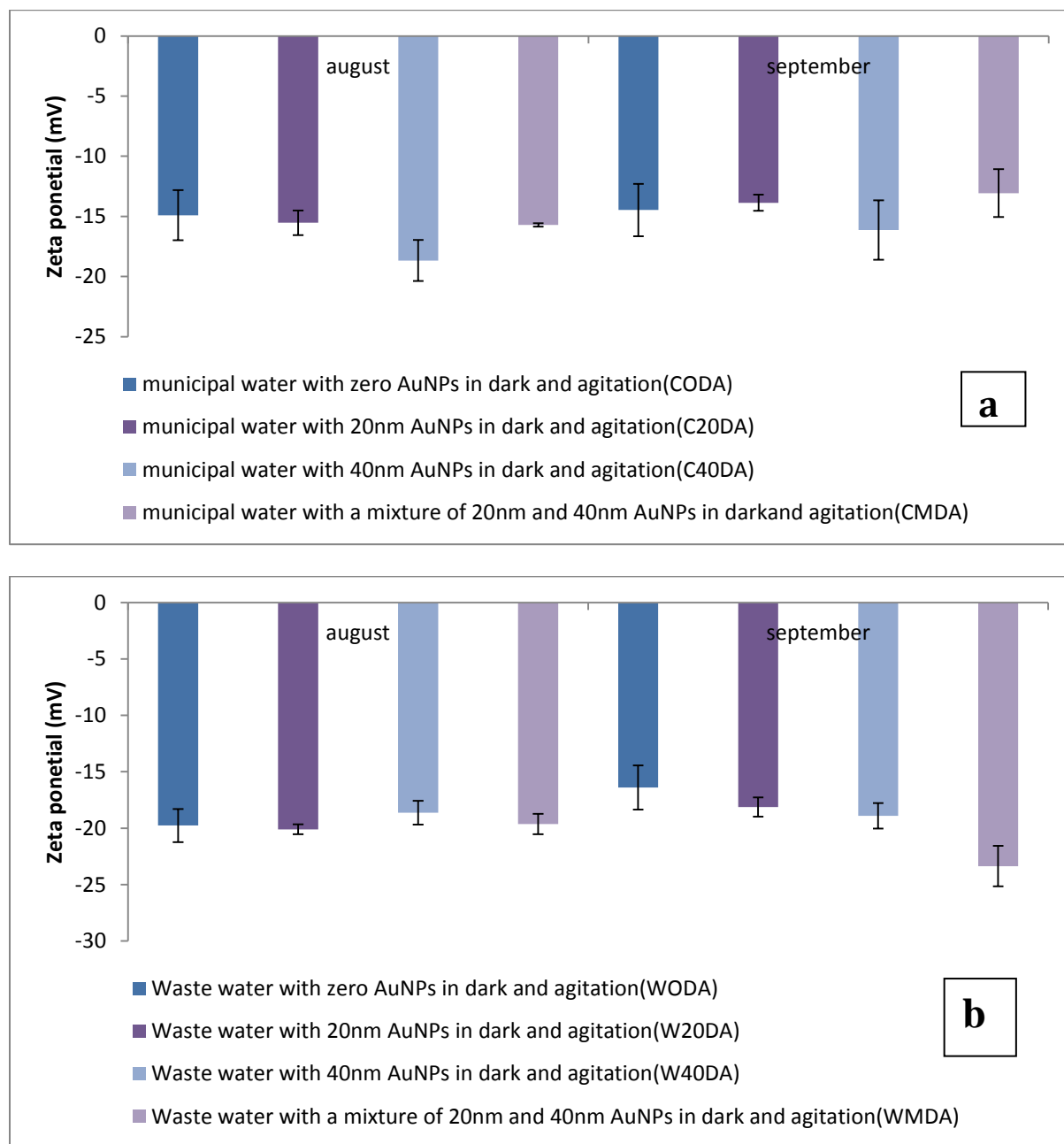


Figure 16: (a) Zeta potential from municipal water with zero, 20nm, 40nm and a mixture of 20nm and 40nm Au-NPs. (b) Zeta potential from waste water with zero, 20nm, 40nm and a mixture of 20nm and 40nm Au-NPs under aerobic, dark conditions with agitation for two months period.

The municipal water with Au-NPs kept in different environments, i.e light, light and agitation, dark, dark and agitation, all under anaerobic condition, retained the unstable particles (ranging between -10.5 mV and -13.8 mV) over a period of two months (Figure 17a). The waste water with Au-NPs in light, light and agitation, all under anaerobic condition, also maintained the unstable particles (ranging between -10.9 mV and -15.1 mV) over a period of two months (Figure 17b).

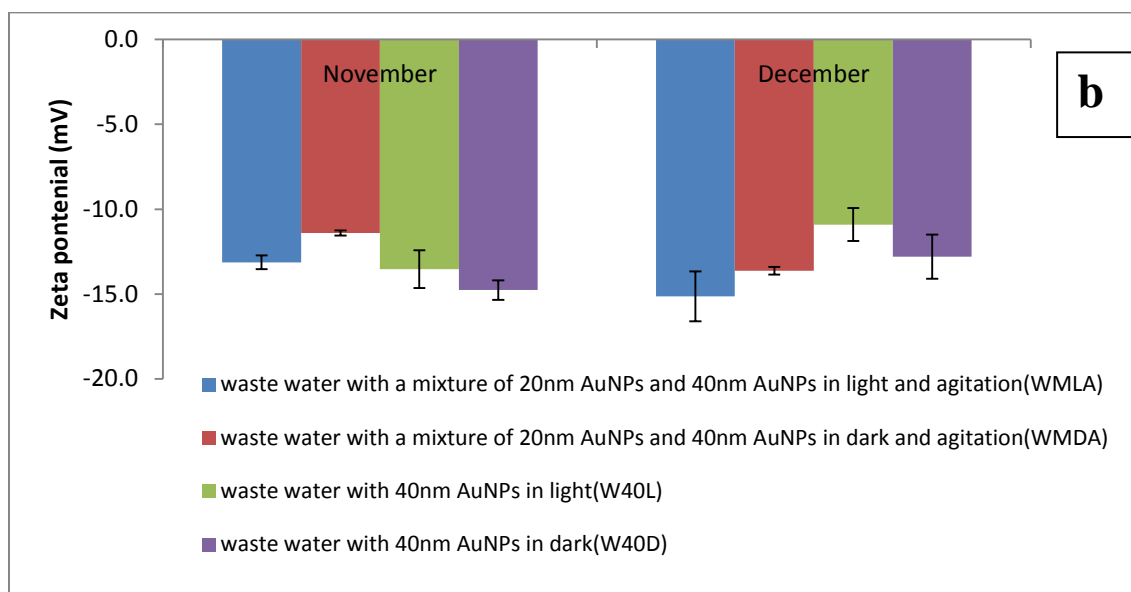
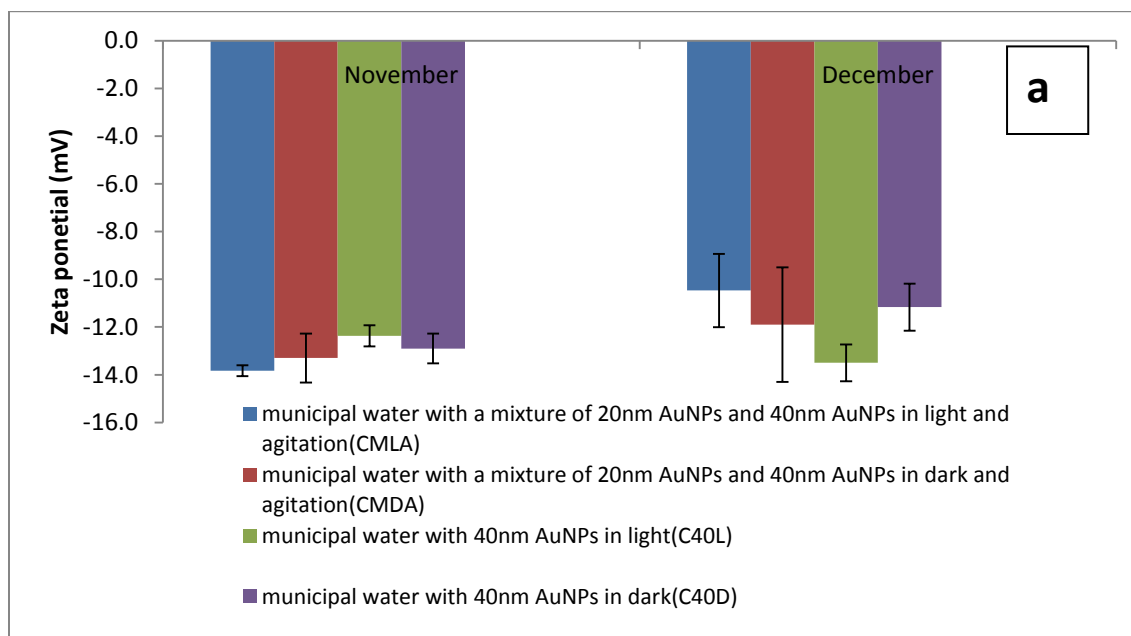


Figure 17: (a) Zeta potential from municipal water with a mixture of 20nm and 40nm Au-NPs in light and agitation, a mixture of 20nm and 40nm Au-NPs in dark and agitation, 40nm Au-NPs in light and lastly 40nm Au-NPs in dark. All samples kept under anaerobic condition. (b) Zeta potential from waste water with a mixture of 20nm and 40nm Au-NPs in light/dark and agitation, a mixture of 20nm and 40nm Au-NPs in dark and agitation, 40nm Au-NPs in light and 40nm Au-NPs in dark. All samples kept under anaerobic condition.

The gold nanoparticles (Au-NPs) in municipal drinking water and waste water exhibited negative variation in zeta potentials (Figures 13 to 17). The zeta potential indicates the stability of gold nanoparticles thereby predicting the interaction between nanoparticles and biological cells (Zhang et al., 2008). The municipal drinking water under aerobic and light conditions (no agitation) had Au-NPs charged between -14 mV and -18 mV for a period of two months (Figure 13a). The waste water with 20nm Au-NPs and waste water with a mixture of 20nm and 40nm Au-NPs in aerobic and light conditions (no agitation) exhibited zeta potential of -29.1 mV and -27.8 mV respectively on the first month (Figure 13b). On the second month, their charges were decreased in magnitude to -19.7 mV and -20.8 mV (Figure 13b). Waste water with zero and 40nm Au-NPs maintained the zeta charge of around -20 mV for the period of two months. The decrease in zeta potential (positive side) showed the loss of stability of nanoparticles.

Citrate was used as a capping agent for stabilizing Au-NPs supplied for the experiment by Mintek. Stability of nanoparticles can be assessed by determining the surface charge and solubility of particles (Hitchman et al., 2013). Citrate weakly binds to nanoparticles and can readily be displaced by a range of molecules including thiols, amines, polymers, antibiotic and proteins (nanocomposix.com). Municipal waste water became more acidic with incubation time in this study (see pH Figure 2b), which also suggests that the carboxyl group were protonated and the zeta potential is reduced resulting in destabilized nanoparticles that might result in aggregation (nanocomposix.com). Thumb rule suggests that the zeta potential greater than 30 mV and/or less than -30 mV is approximately required to maintain dispersion of particles by charge stabilization alone (Hitchman et al., 2013).

5.4 Conclusion

All Au-NPs in municipal water samples had zeta potential charge ≤ 20 mV which is in the unstable region according to the thumb rule. Natural organic matter (NOM) has the ability to bind with metals and organic compounds to reduce their bioavailability. Municipal water has low levels of natural organic matter to stabilize particles. Waste water has the high levels of natural organic matter and other pollutants that can easily displace citrate and bind to nanoparticles. This has been displayed by waste water in light and agitation with and without Au-NPs (Figure 14b). On the first month of incubation, the municipal waste water in light and agitation, with and

without Au-NPs had a charge of -29 mV and were considered stable. On the second month, they lost their stability. This might be due to the displacement of the capping agents and NOM by other pollutants facilitated by agitation. This study shows that Au-NPs released into waste water might end up losing their stability and thus affect their reactivity. Reactive nanoparticles can interact with any other pollutants in the waste water which might even suggest the possible formation of other toxic products.

Chapter 6: The Size and Morphology of Gold nanoparticles using Dynamic Light Scattering (DLS) and Transmission electron Microscopy (TEM) in Aqueous phases

Abstract

Dynamic light scattering (DLS), was used to measure the hydrodynamic sizes of Au-NPs that were disposed into the water for the period of two months. This method can be used to predict the extent of aggregation and /or agglomeration of particles in water. Transmission electron microscope (TEM) was used to determine the actual size and morphology of the nanoparticles. The DLS and TEM data was compared and did not show any possible agglomeration or aggregation of Au-NPs after their disposal into the water systems.

6.1 Introduction

Dynamic light scattering (DLS), also called photon correlation spectroscopy or quasi-elastic light scattering, measures the sizes of suspended particles within a liquid. Particles in the liquid randomly moves around in the process called Brownian motion (nanocomposix.com). As particles moves, larger particles move slower while smaller particles moves faster. Particles in a liquid solution scatter light in correlation with the speed that they are moving. DLS determine the hydrodynamic diameter of particles which include the electrostatic double layer around particles (Hotze et al., 2010). Another technique, the transmission electron microscope was used to determine the size and morphology of the gold nanoparticles.

The Dynamic light scattering (DLS) technique determines the hydrodynamic diameter of colloidal particles. It is used to study the distribution of particles and the extent of agglomeration of particles that occurs during the experiment (Garcia et al., 2012). DLS determine the hydrodynamic diameter of a particle (in d.nm) while TEM micrographs gives a true radius of a particle (Lim et al., 2013). Zeta potential measurements allow the study of the surface properties and any change in the surface of particles. This technique studies the stability of nanoparticles and their surface charge when they are electrostatically stabilized (Garcia et al., 2012).

6.2 Materials and Methods

6.2.1 Dynamic light scattering (DLS)

The DLS analysis were analysed in the same procedure as zeta potential. Cuvettes were carefully filled with the sample to avoid formation of bubbles in the cuvette. Filled cuvettes were put in the cuvette slot of the zetasizer Nano ZS systems (Malvern instruments, 2012) machine, operating at light source wavelength of 532 nm and a fixed scattering angle of 173° for detection. The sample was pre-warmed for 120 minutes before running the test. The results were recorded in triplicates.

6.2.2 Transmission electron microscope (TEM) and EDX

The HRTEM features Joel JEM-2100F Field Emission Electron Microscope, providing best image quality and a maximum analytic resolution of 200 kV with a probe size under 0.5 nm. It is equipped with scanning transmission electron microscope (STEM), energy dispersive x-ray spectrometer (EDAX) and CCD cameras. The same sample prepared for STEM was used to run the EDX part of that sample. The filter paper was placed on an empty Petri dish. The filter paper was graduated into equal spaces by drawing lines across through the centre. Copper grids were carefully placed onto the spaces on the prepared filter paper in petri dish. Samples drops were placed on the grids. Waste water samples were collected from the sediments. Municipal drinking water samples were centrifuged for 30 minutes at 15000 rpm. The supernatant was discarded and the sediment was decanted and transferred onto the relevant grids. The prepared samples were then examined under the microscope by the specialized personnel at Mintek. The TEM pictures were used to measure the diameter of particles to obtain the average particle size.

6.2.3 Data analysis

The *MS Excel 2010* was used to draw the DLS graphs and carried out one factor ANOVA statistical tool (Garcia et al., 2012). TEM pictures were used to measure the radius of gold particles. The TEM results were discussed in comparison with their DLS results.

6.3 Results and discussion

The Dynamic light scattering (DLS), technique was used to understand the morphology of the particles and whether there could be any changes caused by their exposure to pure and waste water systems. The DLS results were displayed on the Figures 18 to Figure 22. The changes in the particle size of the Au-NPs were confirmed by transmission electron microscopy (TEM) (Pictures 2 to 22). The DLS technique is used to determine the hydrodynamic size of gold nanoparticles based on modulation of scattered light, since larger nanoparticles have a slower Brownian motion and thus scatter more light than smaller nanoparticles (nanocomposix.com). Under aerobic and light conditions (no agitation) the waste water samples with 20nm and 40nm Au-NPs increased their particle size from August to September (Figure 18a). The samples with a mixture of 20nm and 40nm Au-NPs did not increase during the same period. Au-NPs in municipal drinking water remained below 500 d.nm except municipal drinking water with a mixture of 20nm and 40nm Au-NPs which increased to 523 d.nm (Figure 18 b).

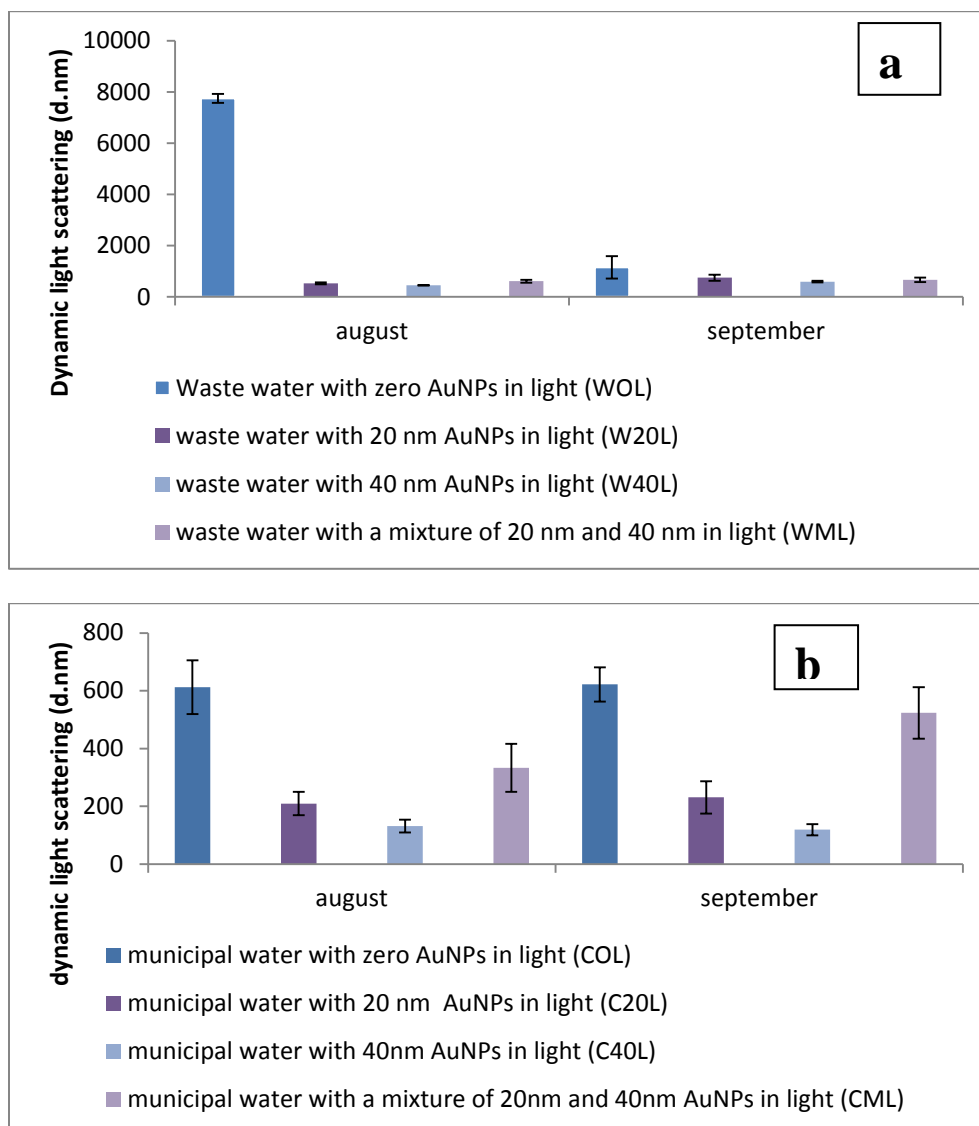
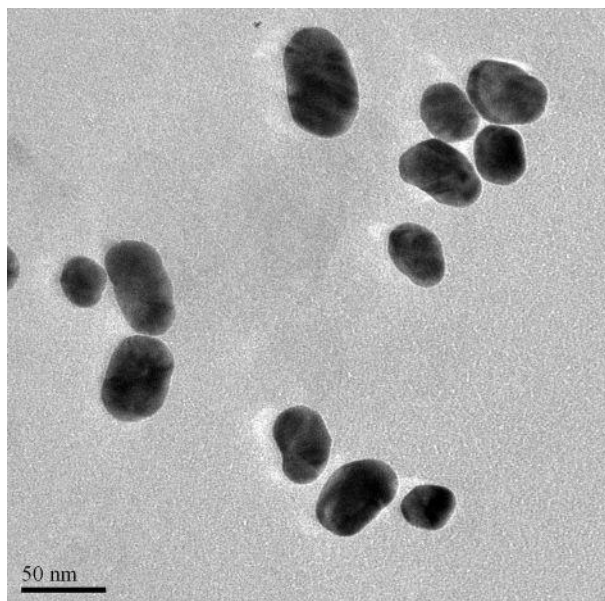
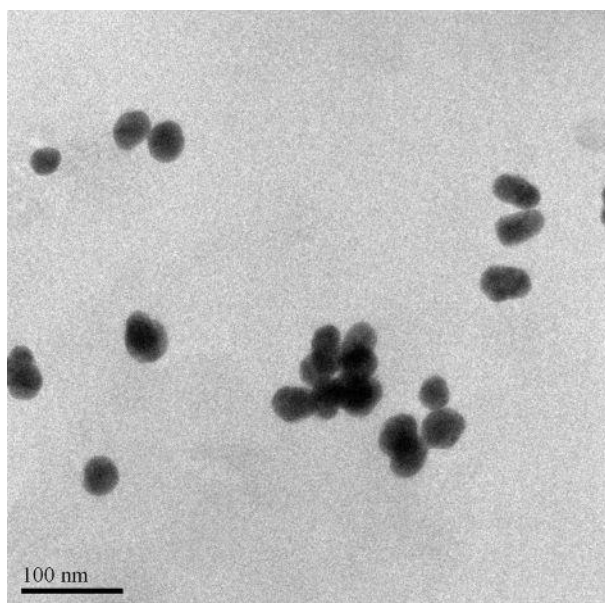


Figure 18: Dynamic light scattering under aerobic, light (no agitation) conditions of: (a) waste water with zero, 20nm, 40nm and a mixture of 20nm and 40nm Au-NPs. (b) Municipal drinking water with zero, 20nm, 40nm and a mixture of 20nm and 40nm Au-NPs.



Picture 2: municipal waste water mixed with 40nm Au-NPs kept in light for one month (W40LR2)

Particles on the picture 2 ranged between 27.8 nm and 63.9 nm. This makes an average particle size of 45.8 ± 18.05 nm. The observations of TEM (picture 2) presents the slightly larger particles than the original 40nm Au-NPs.

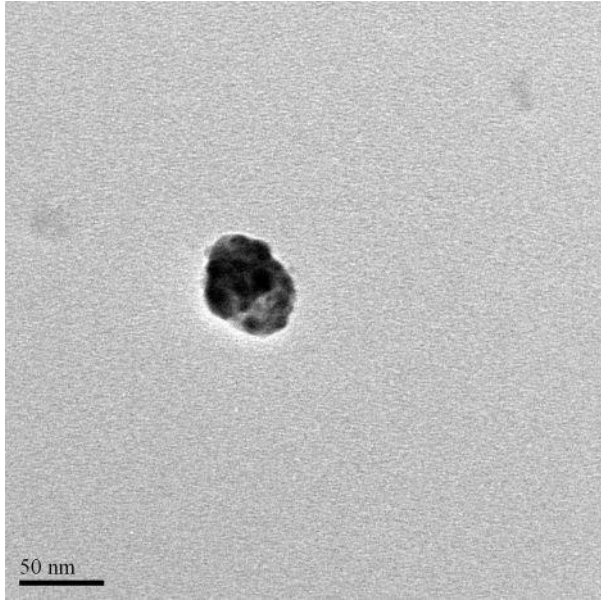


Picture 3: Municipal waste water mixed with 40nm Au-NPs in light for a period of two months (W40LR3).

After two months of incubation of waste water with 40nm Au-NPs in light, particles ranged from 40 ± 8.6 nm (picture 3). The average size of these particles suggests the preservation of the original size of 40nm added to waste water.

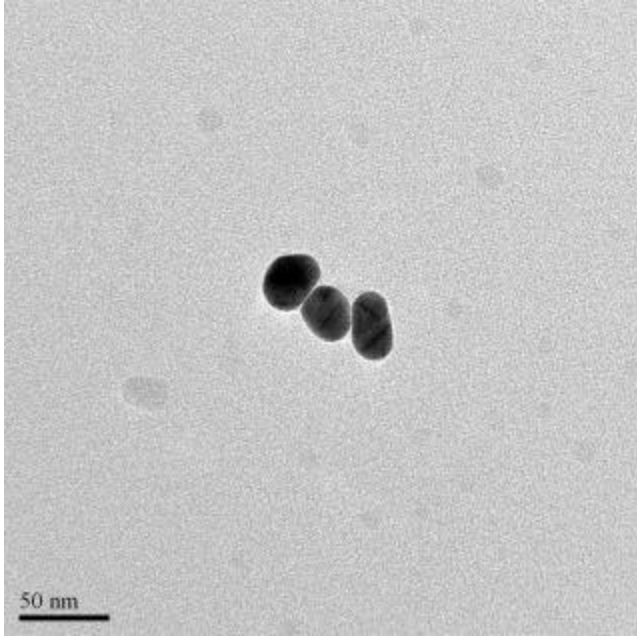
The DLS measurements of waste water with 40nm Au-NPs kept in light was 457 d.nm and an average TEM size of 45.8 nm in the second month which is slightly greater than the average of 40nm in September. In contrast, the DLS for the same sample after two months in September was increased to 599 d.nm (Figure 18 a).

The DLS measurements of municipal waste water with 40nm Au-NPs kept in light suggest the possible enlargement/aggregation of particles on the second month even though the TEM results do not corroborate the DLS as the average particles was 40nm size in September. Under aerobic and light conditions (no agitation) the municipal drinking water samples with 20nm and 40nm Au-NPs did not change size during same period according to DLS data (Figure 18b). TEM picture of municipal drinking water with 40nm Au-NPs showed an enlarged particle (Picture 4) below. However during the same period, municipal drinking water with a mixture of 20nm and 40nm Au-NPs showed an increase in their particle size from 333 d.nm to 523 d.nm (Figure 18b). The TEM picture of a mixture of 20nm and 40nm do not support the DLS results (picture 5).



Picture 4: Municipal drinking water with 40nm Au-NPs in light for a month (C40LR2)

Only one particle was examined on the TEM microscope and was larger than the expected particle size of 63.3 nm which was greater than 40nm (Picture 4). This might suggest the possible enlargement of the nanoparticles. Municipal drinking water had to be centrifuged to concentrate Au-NPs in order to find particles on the microscopic slide. At some stage it was even difficult to find any particle on the slide. This may be attributed to the small amount of Au-NPs used in the study to avoid over exaggeration of the environmental condition with regard to nanoparticles contamination.



Picture 5: Municipal drinking water with a mixture of 20nm and 40nm Au-NPs in light after two months (CMLR3)

Municipal drinking water with a mixture of 20nm and 40nm Au-NPs in light after two months of incubation showed a range of particles from 30nm and 38 nm (picture 5). This range of particles seems to have excluded the 20nm Au-NPs. This might have been caused by the difficulty in concentrating particles in municipal drinking water samples for microscopic preparations.

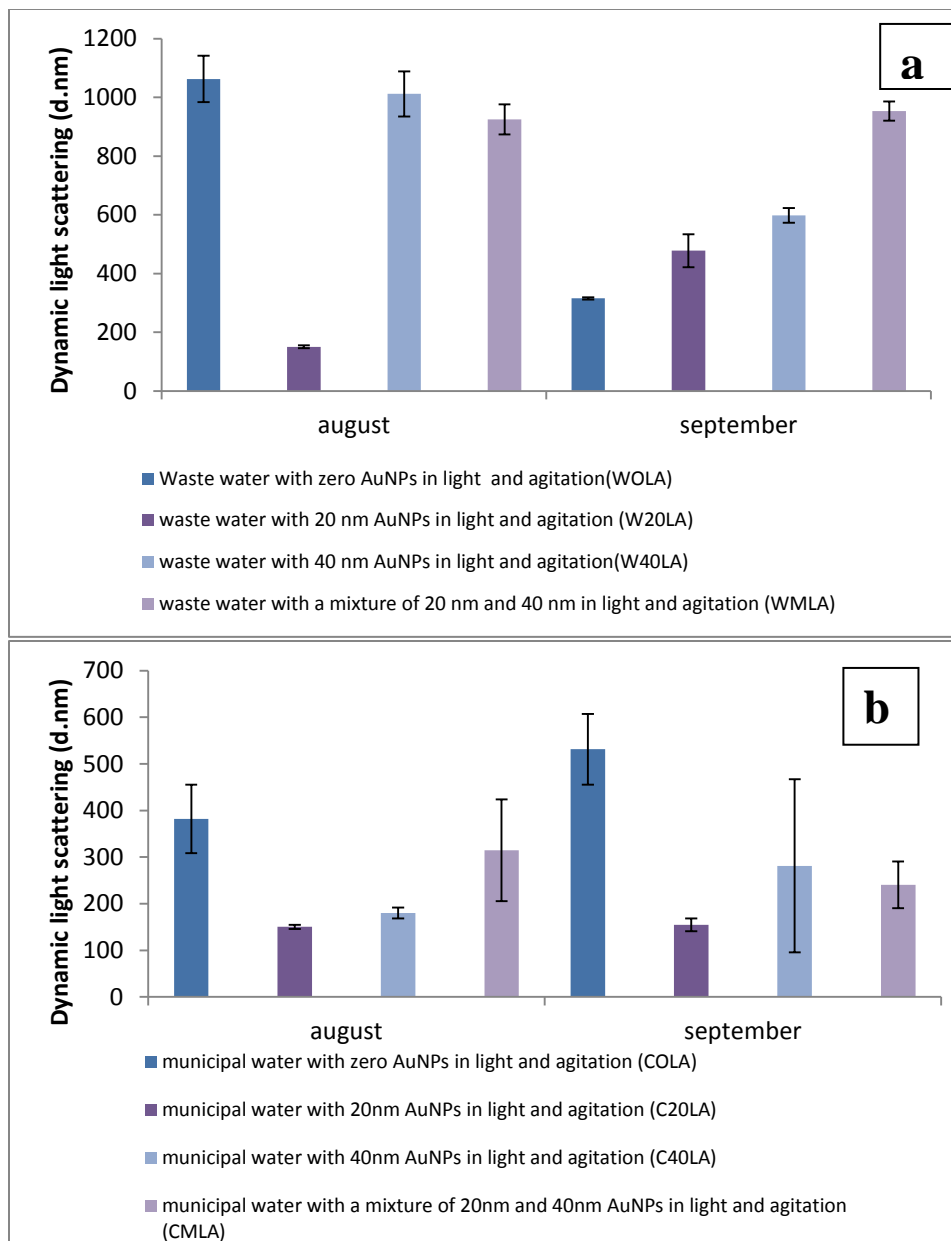
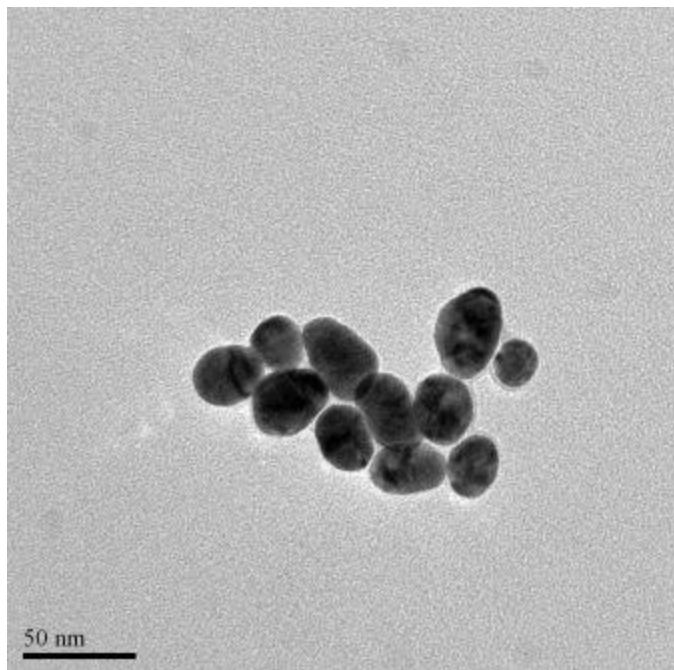
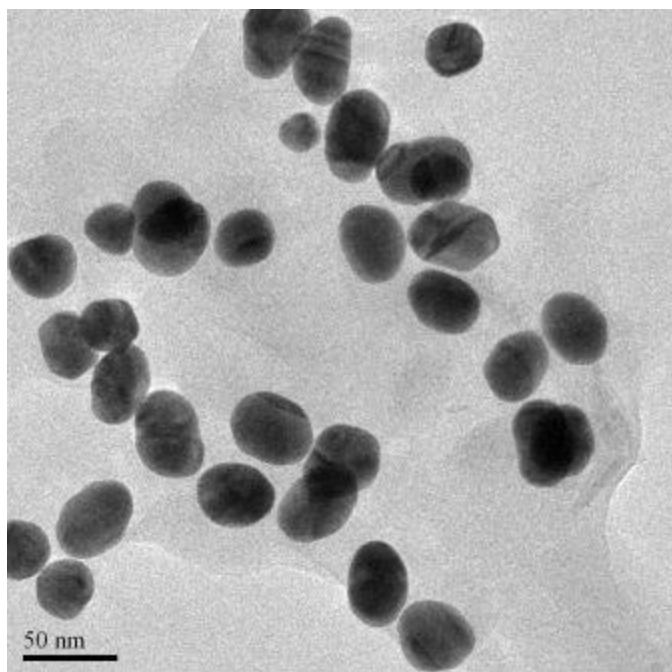


Figure 19: Dynamic light scattering under aerobic, light and agitation conditions of: (a) municipal waste water with zero, 20nm, 40nm and a mixture of 20nm and 40nm Au-NPs. (b) Municipal drinking water with zero, 20nm, 40nm and a mixture of 20nm and 40nm Au-NPs.



Picture 6: Municipal drinking water with a mixture of 20nm and 40nm Au-NPs kept in light and agitation for two months (CMLA aero R3)

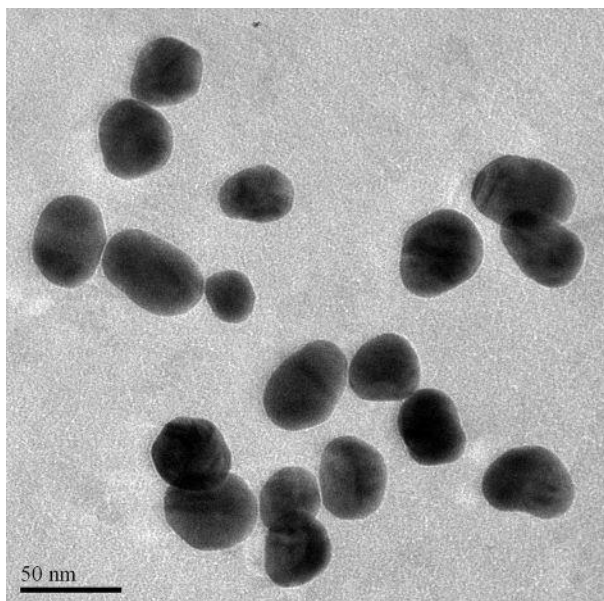
Gold nanoparticles ranged between 21.4 nm and 42.9 nm after the incubation for two months. The DLS (Figure 19b) results might be analysed as in corroboration with the TEM picture 6 as the particle sizes were lower than 400 d.nm. There was no likelihood of any change in particle size in municipal drinking water with a mixture of 20nm and 40nm Au-NPs in light and agitation for two months.



Picture 7: municipal waste water with 40nm Au-NPs in light and agitation for one month (W40LAR2)

Gold nanoparticles in light and agitation kept for a month presented a range of particles sizes between 34.2 ± 12.5 nm (picture 7). This might suggest the possible reduction in size of the particles in light an agitation. The DLS results shows the highest particle size (1012 d.nm) on the first month and a reduced size of 598 d.nm on the second month (Figure 19a). This may suggest the aggregation of particles in the beginning of mixing waste water with 40nm Au-NPs but the TEM exhibit the opposite.

Under aerobic and light conditions with agitation the waste water samples with 20nm Au-NPs increased their particle size but still remain below 500 d.nm in two months (Figure 19a). Under the same conditions and time period, waste water samples with 40nm Au-NPs maintained the particle sizes above 500 d.nm for the duration of the experiment. A mixture of 20nm and 40nm Au-NPs maintained the DLS greater than 800 d.nm for two months.



Picture 8: municipal waste water with 40nm Au-NPs kept in light and agitation the two month period (W40LAR3).

The TEM observations of gold nanoparticles showed the average of 38.9 ± 13.0 nm which may not suggest any alteration in terms of particle size (Picture 8). The average TEM particle size in waste water in light and agitation in the first month (Picture 7) was slightly lower than in the second month (Picture 8).

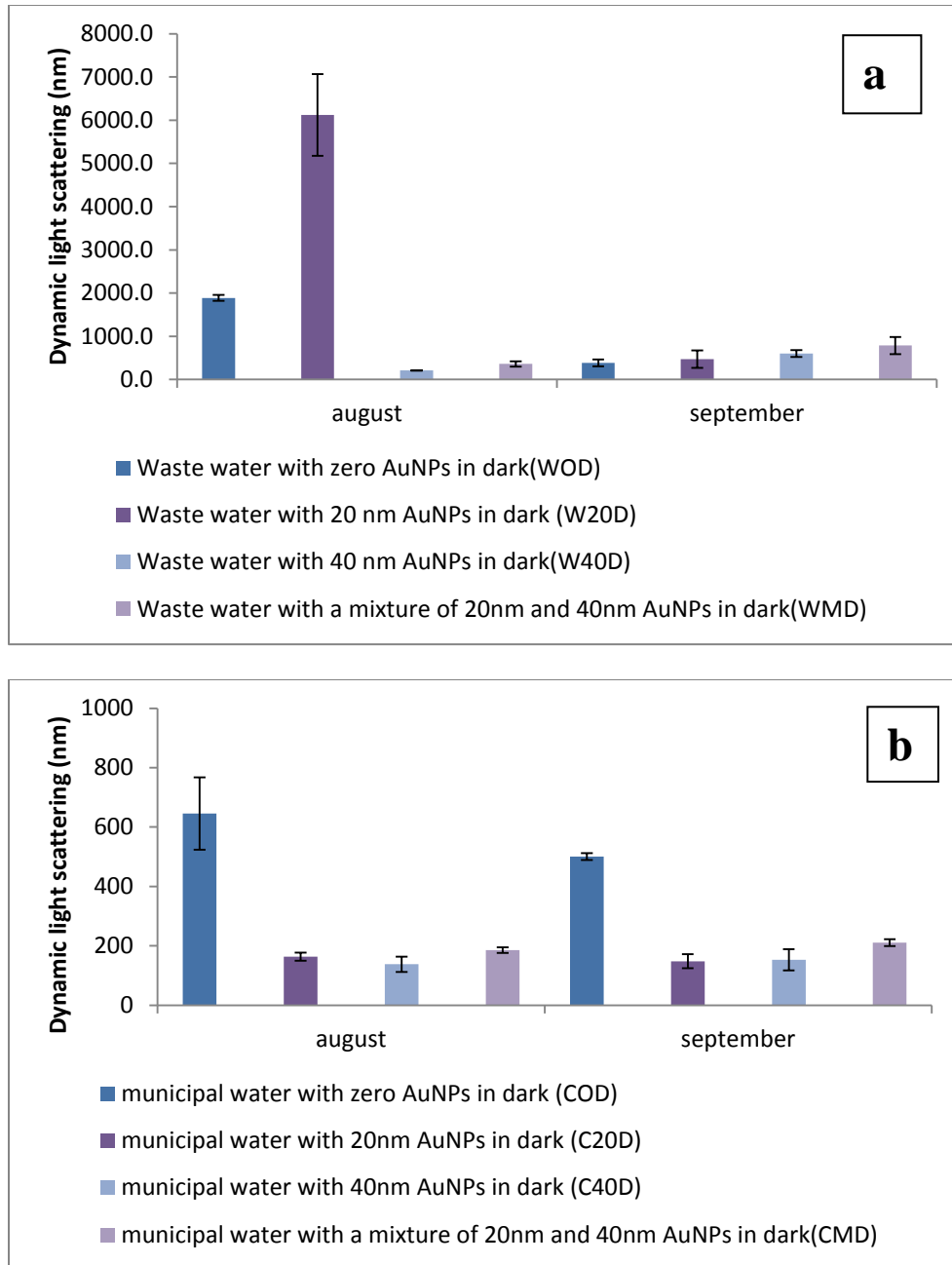
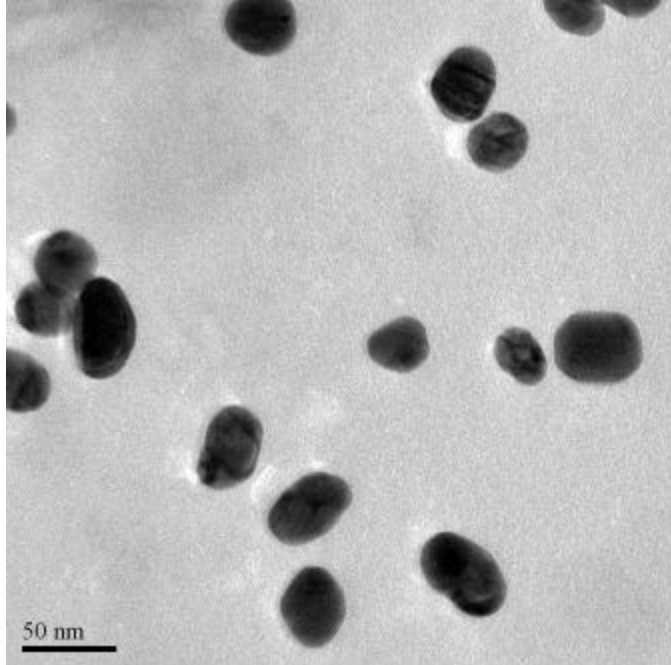


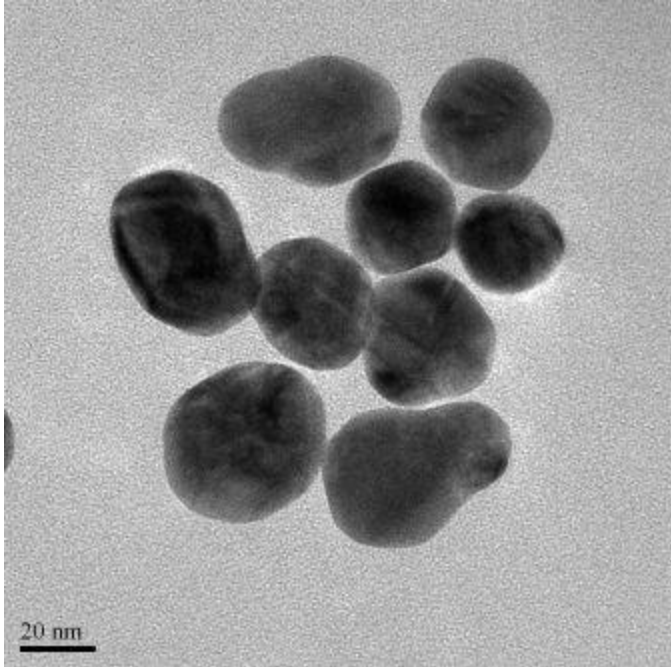
Figure 20: Dynamic light scattering under aerobic, dark (no agitation) conditions of: (a) municipal waste water with zero, 20nm, 40nm and a mixture of 20nm and 40nm Au-NPs. (b) Municipal drinking water with zero, 20nm, 40nm and a mixture of 20nm and 40nm Au-NPs.



Picture 9: TEM picture of municipal drinking water mixed with 40nm Au-NPs kept under dark conditions for one month (C40DR2)

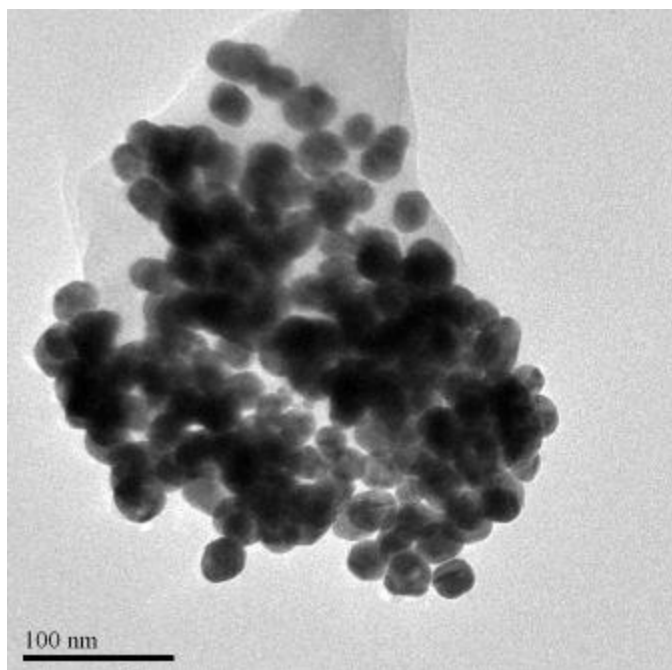
The nanoparticles' size ranged from 38.65 ± 6.85 nm (Picture 9). There is no sign of any alteration of particles size after contact with waste water away from light.

Under aerobic and dark conditions (no agitation) the municipal drinking water samples with 20nm Au-NPs and with 40nm Au-NPs and the mixture of 20nm and 40nm Au-NPs maintained consistent particle sizes lower than 500 d.nm from August to September (Figure 20b).



Picture 10: Waste water with 40nm Au-NPs away from light (W40D)

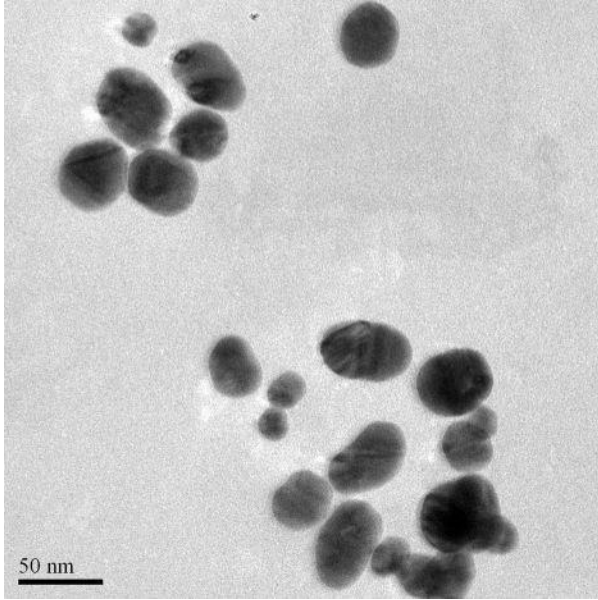
Particles ranged between 40.4 ± 11.2 nm after a month of incubation. The TEM (Picture 10) does not suggest any change in the particles size during the experiment. In contrary, the DLS shows an increase in hydrodynamic particle size to 600 d.nm (Figure 20a), which may suggest the possible particles agglomeration. TEM measures the exact size of the particle while DLS determine the hydrodynamic size of the particle. The increase in DLS to above 500d.nm can be considered as a possibility of particles agglomeration.



Picture 11: TEM picture of municipal drinking water mixed with 40nm Au-NPs kept under dark conditions for two months (C40DR3)

Gold nanoparticles size was ranging between $31.1 \pm 10.4\text{nm}$ (Picture 11). The TEM picture shows a sheath like membrane around particles but DLS results do not support the assumption that it might be aggregation; hence the size remained from 138d.nm in August to 152.7d.nm in September (Figure 20b). The grouping together of particles might have been caused by centrifugation of samples to concentrate them for TEM analysis.

There might be a possible slight reduction of gold particle's sizes in a period of two months when 40nm Au-NPs in municipal drinking water which were kept away from light.



Picture 12: The TEM picture of waste water with a mixture of 20 and 40nm Au-NPs kept away from light for a month (WMDR2)

The nanoparticles sizes ranged from 18.5nm to the largest of 55.6nm (picture 12). Picture 12 does not show any probability of size alteration. Under anaerobic condition, waste water with 40nm Au-NPs in dark was the only sample that presented an increase to 642d.nm (Figure 22a) which is greater than 500d.nm.

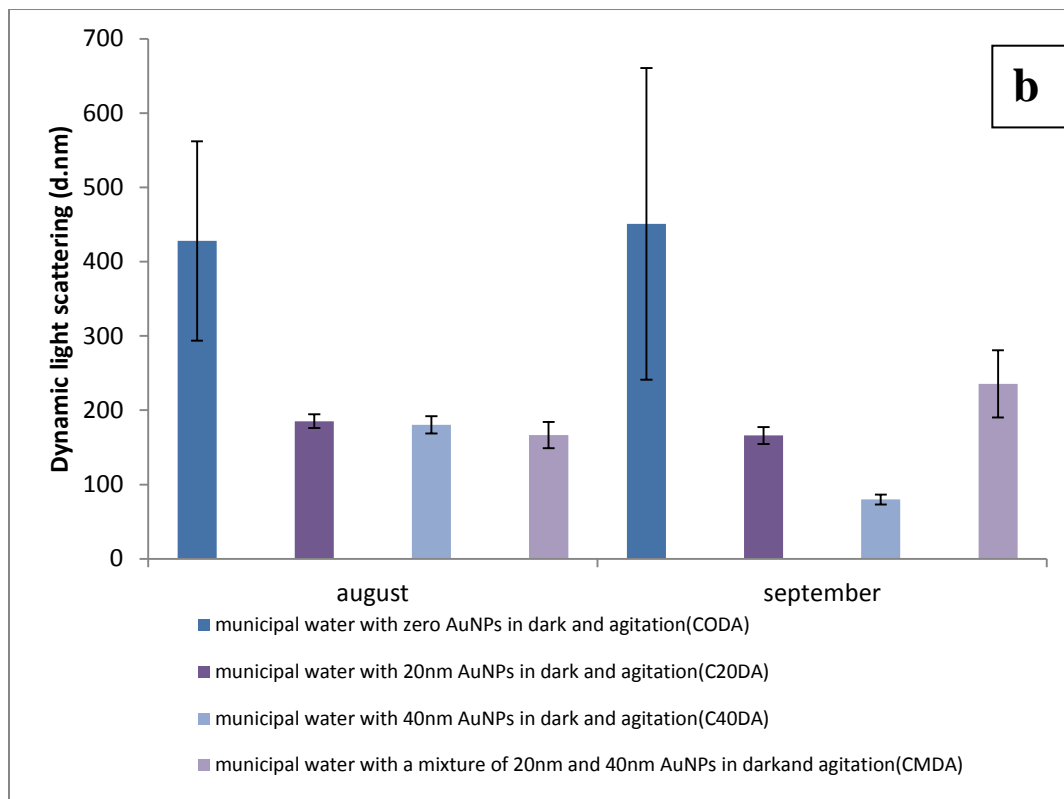
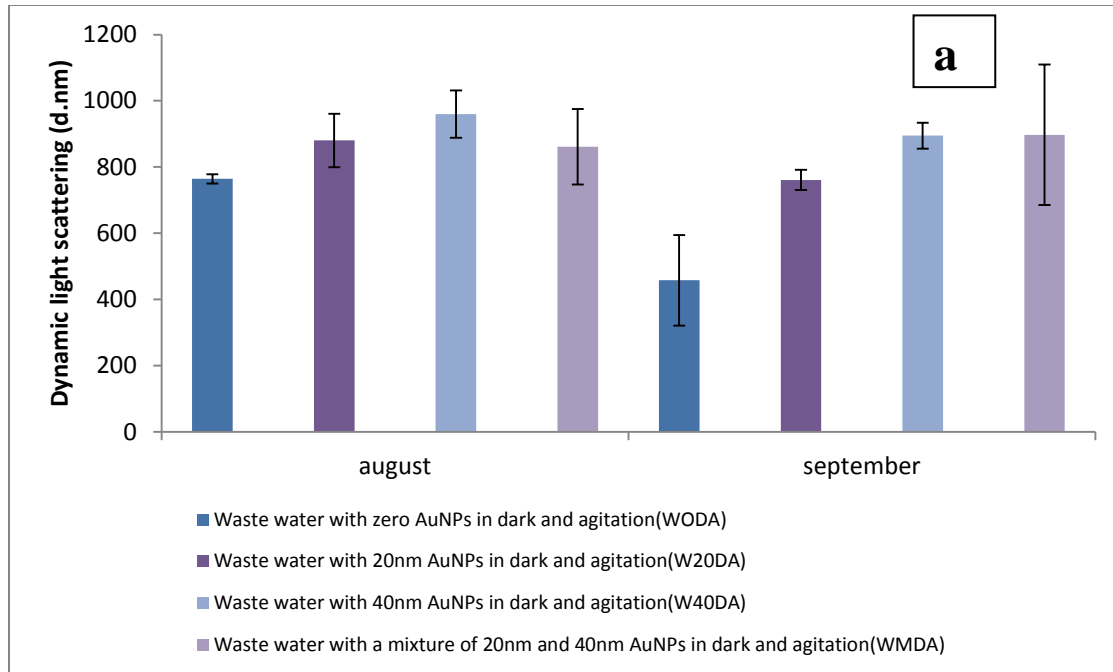
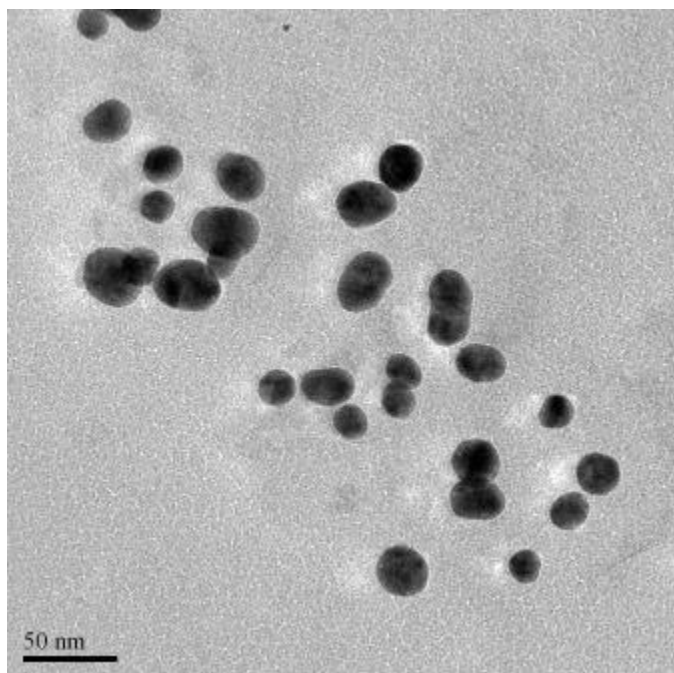


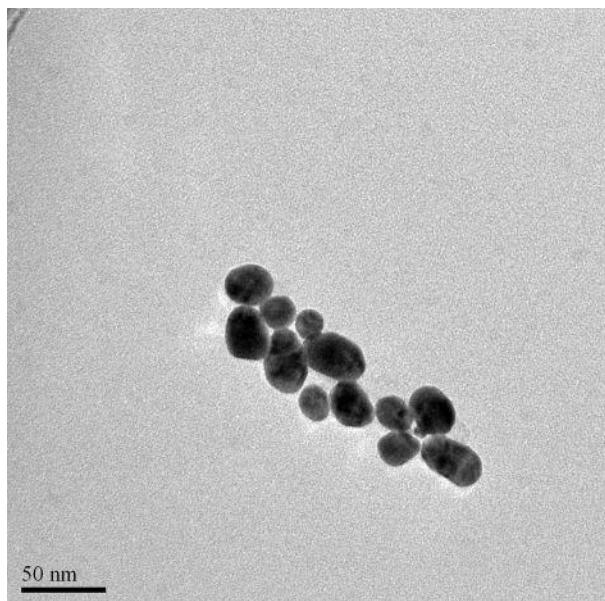
Figure 21: Dynamic light scattering under aerobic, dark conditions with agitation of: (a) municipal waste water with zero, 20nm, 40nm and a mixture of 20nm and 40nm Au-NPs. (b) Municipal drinking water with zero, 20nm, 40nm and a mixture of 20nm and 40nm Au-NPs.



Picture 13: Waste water with 20nm Au-NPs under aerobic dark and agitation condition after two months of incubation (W20DAR2)

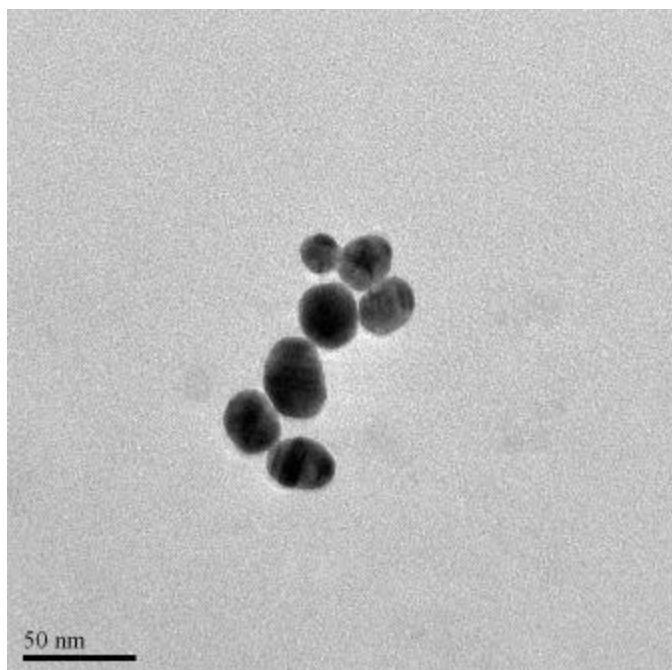
Waste water with 20nm Au-NPs under aerobic condition in dark and agitation after two months of incubation presented gold nanoparticles range 26.65 ± 8.35 nm (picture 13). This result might suggest the possible change in size of some gold nanoparticles during the experiment.

Under aerobic and dark conditions with agitation, waste water samples with 20nm Au-NPs and 40nm Au-NPs and with a mixture of 20nm and 40nm Au-NPs all maintained the greater than 500 d.nm size in two months (Figure 21a).



Picture 14: Municipal drinking water with a mixture of 20nm and 40nm Au-NPs on agitation away from light for a month (CMDAR2)

Municipal drinking water with a mixture of 20nm and 40nm Au-NPs in the dark and agitation after a month of incubation, presented the sizes ranging between 16nm and 40nm (Picture 14). These particle sizes do not suggest if there was any alteration in particle size during the experiment. According to DLS data, all experiments involving municipal drinking water mixed with Au-NPs in dark and agitation did not have any significant change in size as they (Au-NPs) retained their hydrodynamic sizes below 500 d.nm (Figure 21b).



Picture 15: Municipal waste water with a mixture of 20nm and 40nm Au-NPs under anaerobic condition in dark and agitation for two months (WMDA anaerobic R2)

Municipal waste water with a mixture of 20nm and 40nm Au-NPs under anaerobic condition in dark and agitation for two months presented particles ranging between 17.1 nm and 37.1 nm (picture 15). This might suggest a slight reduction in size of the 40nm Au-NPs.

The municipal waste water samples with a mixture of 20nm and 40nm Au-NPs in light and agitation, 40nm Au-NPs in light and 40nm Au-NPs in dark, all these had an increase in size (Figure 22a) under anaerobic conditions. The waste water samples with a mixture of 20nm and 40nm Au-NPs in dark and agitation had an abnormally/ invalid high value on the second month. The waste water with 40nm Au-NPs in dark increased in particle size drastically in two months (Figure 22a). All municipal drinking water samples with Au-NPs maintained their sizes below 500 d.nm (Figure 22b)

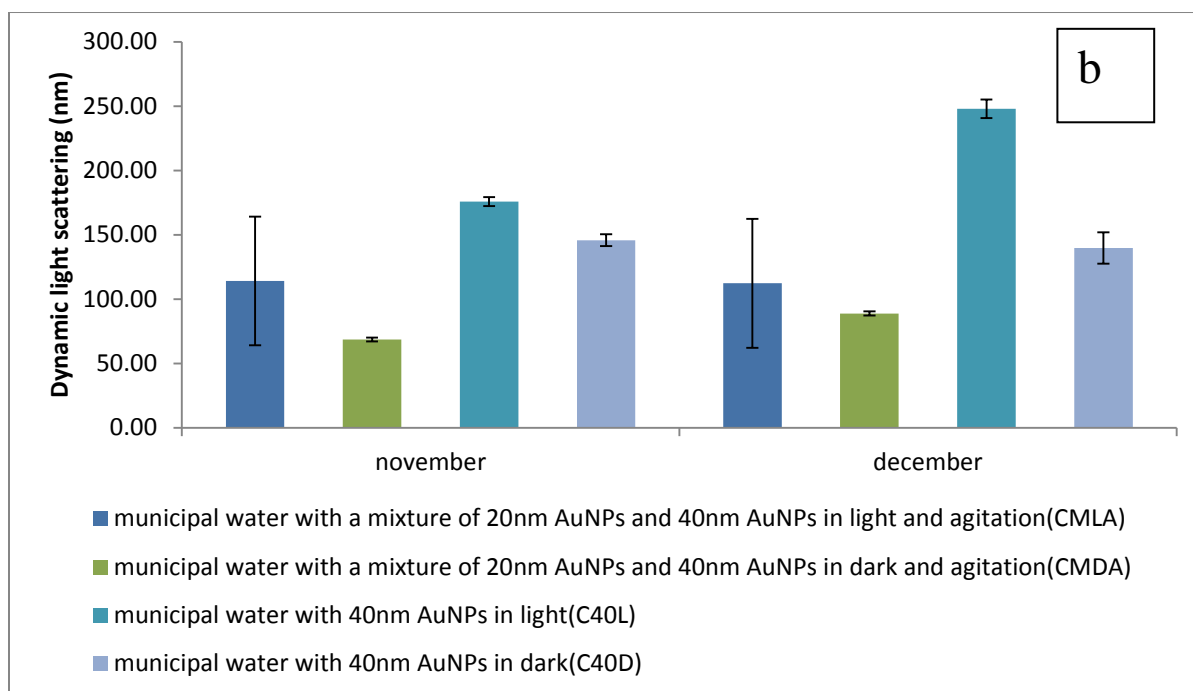
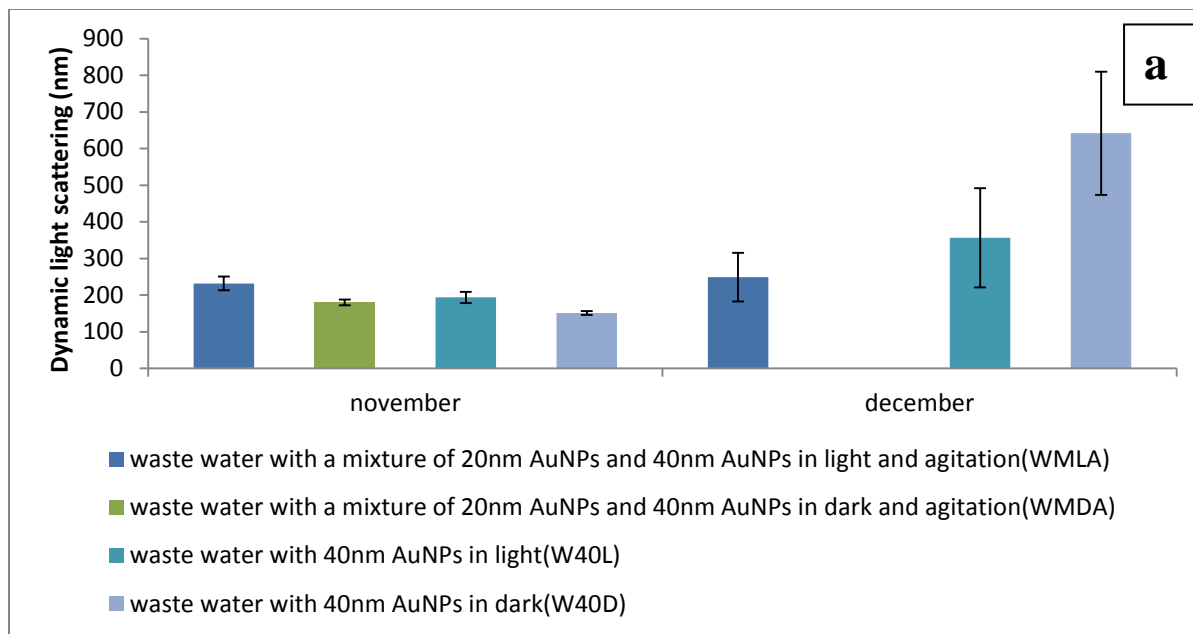
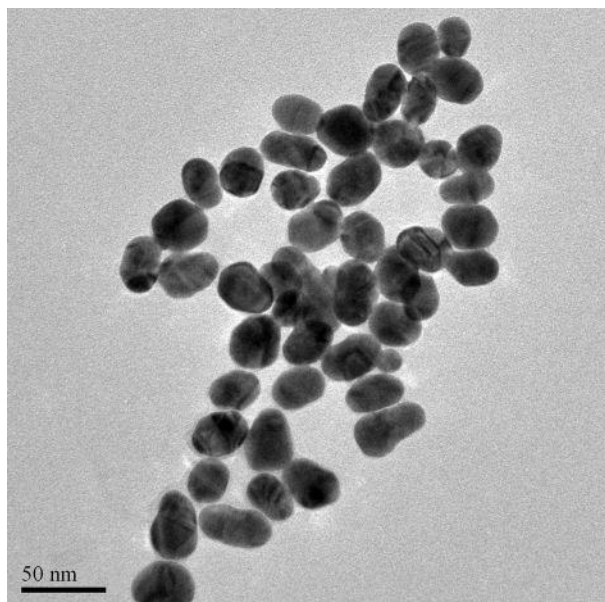
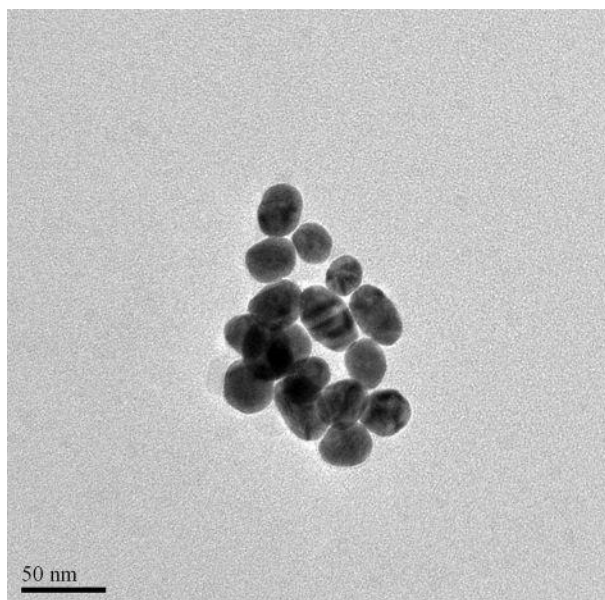


Figure 22: Dynamic light scattering under anaerobic of (a)municipal waste water with a mixture of 20nm and 40nm in light and agitation, municipal waste water with a mixture of 20nm and 40nm in dark and agitation, waste water with 40nm Au-NPs in light and waste water with 40nm Au-NPs in dark. (b) Municipal drinking water with a mixture of 20nm and 40nm in light and agitation, municipal drinking water with a mixture of 20nm and 40nm in dark and agitation, municipal drinking water with 40nm Au-NPs in light and municipal drinking water with 40nm Au-NPs in dark



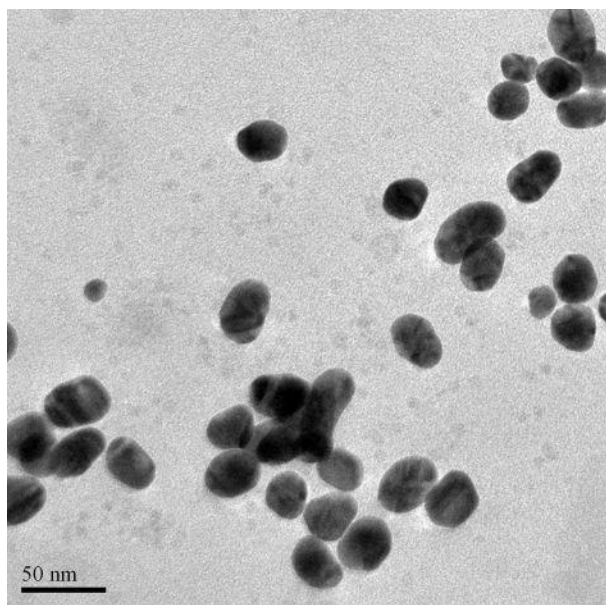
Picture 16: Municipal water was mixed with 40nm Au-NPs under anaerobic condition in light for a month (C40L anaerobic R2)

Gold nanoparticles presented a mean size of 36.12 ± 8.3 nm (Picture 16). This average nanoparticle size does not suggest any possible alteration of gold nanoparticles under light conditions. The DLS results also support the TEM hence size lower than 500d.nm (Figure 22b).



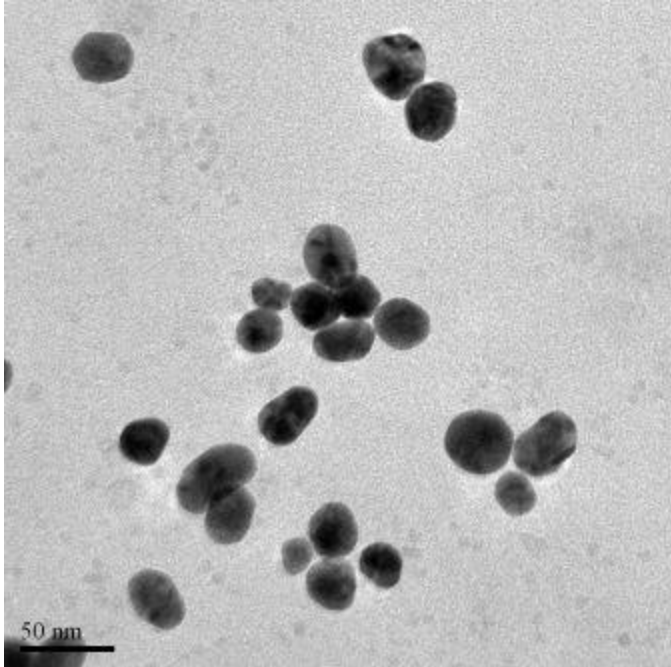
Picture 17: Waste water with 40nm Au-NPs in light under anaerobic condition kept for two months (W40L anaerobic R3)

The municipal waste water with 40nm Au-NPs in light under anaerobic condition kept for two months, presented the particles ranging between 30.8 ± 7.5 nm (picture 17). The DLS of waste water with 40nm Au-NPs in light under anaerobic condition maintained the size smaller than 400d.nm (Figure 22a). The DLS and TEM of waste water with 40nm Au-NPs in light and anaerobic seem to agree. There was probably no effect of anaerobic condition to Au-NPs in waste water with 40nm Au-NPs in light.



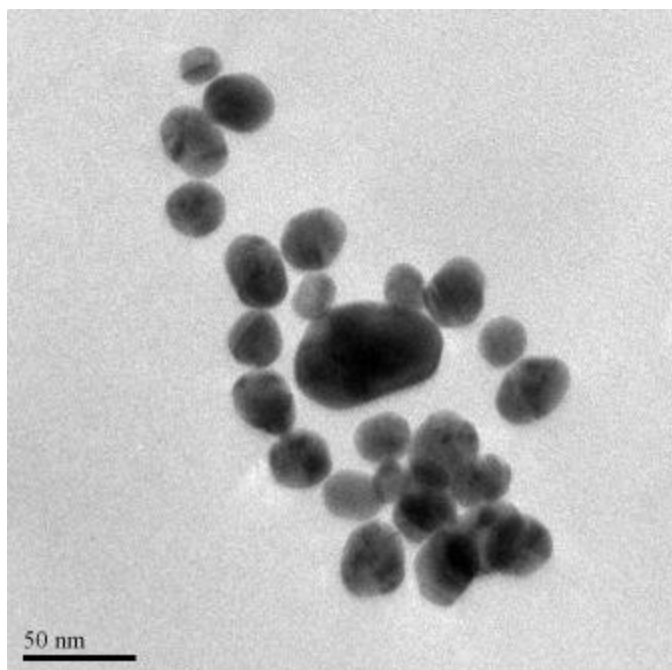
Picture 18: municipal waste water with 40nm Au-NPs in dark under anaerobic condition kept for one month (W40D anaerobic R2)

Waste water with 40nm Au-NPs in dark under anaerobic condition showed the mean particle range of 31.9 ± 16.4 nm (Picture 18). The mean particle size is lower because of some few very small particles. These extremely small particles might have been synthesized by some organisms that are capable of producing gold nanoparticles.



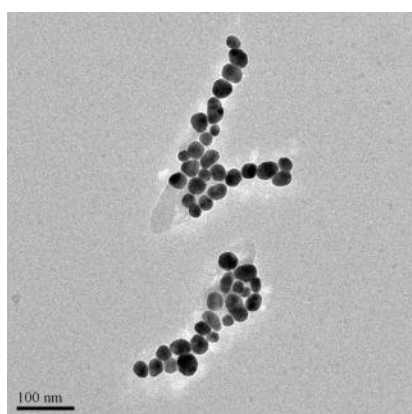
Picture 19: municipal waste water with 40nm Au-NPs in dark under anaerobic condition for two months (W40D anaerobic R3)

Waste water with 40nm Au-NPs in dark under anaerobic condition for two months presented particles' sizes that ranged between $35 \pm 13\text{nm}$ (Picture 19). This average size range does not suggest any possible change in the Au-NPs after two months. In contrast, under anaerobic condition, waste water with 40nm Au-NPs in dark was the only sample that presented an increase to 642d.nm (Figure 22a) which is greater than 500d.nm. These results shows contradiction as TEM has no significant change while DLS presented the increase which suggests the possible aggregation.



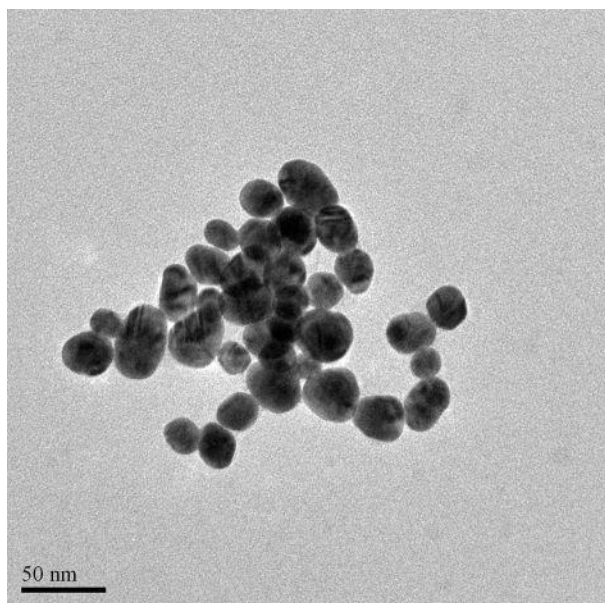
Picture 20: Municipal waste water with a mixture of 20nm and 40nm Au-NPs under anaerobic condition with agitation and kept away from light for one month (WMDA anaerobic R2)

After one month of incubation, particles ranged between 21.4nm and 68 nm (Picture 20). These results might suggest the possible enlargement of nanoparticles. But the DLS results were below 250d.nm on the first month and invalid on the second month (Figure 22a) and thus cannot give a conclusive suggestion.



Picture 21: Municipal water with a mixture of 20nm and 40nm Au-NPs under anaerobic condition in light and agitation after one month (CMLA anaerobic R2)

The municipal drinking water with a mixture of 20nm and 40nm Au-NPs under anaerobic condition in light and agitation after one month of incubation showed particles range between 20nm and 46.7nm (picture 21). This result presents the conservation of original particle's sizes of 20 and 40nm (Figure 22a). The DLS of waste water with a mixture of 20nm and 40nm Au-NPs in light and agitation under anaerobic condition were all maintained below 250d.nm for two month.



Picture 22: Municipal drinking water with a mixture of 20nm and 40nm Au-NPs under anaerobic condition in light and agitation after two months (CMLA anaerobic R3)

Municipal drinking water with a mixture of 20nm and 40nm Au-NPs under anaerobic condition in light and agitation after two months of incubation had particles ranging from 19.4 nm to 41.7nm which might suggest no change of particle sizes (picture 22b). This is supported by DLS results that show sizes below 250d.nm on experiments involving municipal drinking water with Au-NPs.

The municipal waste water contains a variety of chemicals including organic matter, heavy metals, chlorides and pesticides among others bacteria and protozoa (Shon et al., 2007). Some bacteria that can synthesize nanoparticles, for example *Thiobacillus ferrooxidans*, can reduce

ferric ion to ferrous state using sulphur as an energy source (Iravani, 2014). Waste water from Malamulele waste water treatment plant contained high levels of Sulphur (464 mg/l), which favours the growth of bacteria that feeds on Sulphur. Natural organic matter, due to its macromolecular nature, is expected to prevent aggregation of nanoparticles (Hotze et al., 2010). Nevertheless, with some of the microorganism including *E. coli* DH5 α that are capable of synthesizing gold nanoparticles (Iravani, 2014) there is a possible accumulation of nanoparticles in the waste water. Medium with high concentration of nanoparticles will have higher tendency to aggregate and form larger clusters (Lim et al., 2013).

Waste water used contained not only Sulphur (464ppm) as stated above, but also calcium (28ppm), Magnesium (8.2ppm), Chlorides (27.8ppm) which makes it possible to form salts in the waste water. The provided gold nanoparticles for the experimental purpose were stabilized with citrate. Citrate protected gold nanoparticles are susceptible to agglomeration in high salt concentration solution. Citrate stabilized Au-NPs are known to be unstable in electrolytes solution (Jans et al., 2009).

6.4 Conclusion

Dynamic light scattering technique is very sensitive and suitable to determine and monitor the presence of small aggregates of gold nanoparticles. Aggregation is observed with a detection of particles groups with a diameter ≥ 500 nm. The interpretation of DLS data involves the relationship of parameters including particle size, shape, and concentration of particles, polydispersity and surface properties of nanoparticles involved. Then under anaerobic conditions, the particles sizes remained below 500nm except waste water with 40nm Au-NPs in dark which increased to 642nm (Figure 22a). This hydrodynamic size increase does not support the TEM picture 17) which shows an average of 35 nm Au-NPs which is acceptably slightly lower than 40nm. The Municipal drinking water with a mixture of 20nm and 40nm Au-NPs in light was the only scenario that had a size increase to 523nm (Figure 18b). Almost all waste water samples had particles with hydrodynamic sizes of 500nm or more on the first month of mixing with Au-NPs. This might probably suggest some possible tendency of nanoparticles aggregation just after adding gold nanoparticles in the waste water.

Chapter 7: The presence of Au in NanoGold particles

Abstract

The EDX or EDAX was used to confirm the presence of Au⁰ in the municipal drinking water and waste water during the two months' time of the experiment. The study confirmed that the disposed gold nanoparticles into the water systems would exist as Au⁰ and would probably be discharged into the water bodies.

7.1 Introduction

Energy dispersive x-ray spectroscopy (EDX) is a powerful tool to determine the elemental composition of a sample. The lower atomic number elements have few peaks while higher atomic numbered elements have more number of x-ray peaks (<http://www.seallabs.com/how-sem-eds-works.html>). When electrons of the element are excited, they gain more energy and are ejected from their shells and create an electron hole in the inner shell. Electrons from the outer shell lose energy in order to flow down to replace the ejected electrons. The lost energy above produces the x-ray radiation. When an electron from the K-shell is lost and ejected and is replaced by L-shell electron is called K alpha x-ray but when it is replaced by an electron from the M shell, it is called L-beta x-ray (Hafner, 2006). Elements are characterized by their respective number of peaks and positions.

7.2 Materials and Methods

7.2.1 Transmission electron microscope (TEM) and EDX

The HRTEM features Joel JEM-2100F Field Emission Electron Microscope, providing best image quality and a maximum analytic resolution of 200kV with a probe size under 0.5nm. It is equipped with scanning transmission electron microscope (STEM), energy dispersive x-ray spectrometer (EDAX) and CCD cameras. The transmission electron microscope was used to determine the size and morphology of the nanoparticles. The same sample prepared for STEM was used to run the EDX part of that sample. The filter paper was placed on an empty Petri dish.

The filter paper was graduated into equal spaces by drawing lines across through the centre. Copper grids were carefully placed onto the spaces on the prepared filter paper in petri dish. Samples drops were placed on the grids. Waste water samples were collected from the sediments. Municipal drinking water samples were centrifuged for 30 minutes at 15000 rpm. The supernatant was discarded and the sediment was decanted and transferred onto the relevant grids. The prepared samples were then examined under the microscope by the specialized personnel at Mintek.

7.2.2 Data analysis

The EDAX photographs captured by the TEM were observed for the presence of Au⁰ peaks. The positions of peaks on the graphs were recorded on the table.

7.3 Results and discussion

The energy dispersive x-ray spectrometer (EDAX) showed the presence of elements such as C, Cr, Fe and Au in the municipal waste water and no Cr in the municipal drinking water (Figure 26). On interest was the presence of Au⁰ in the municipal drinking and waste water samples (Table 33). The optical absorption peak for Au is 2.2keV (Geethalakshmi and Sarada, 2012) which is in corroboration with the Au peaks at (2.2 -2.4)keV from this study (Figure 23 to 26) were observed.

Table 33: Places where peaks are visible on the EDX graphs

	Energy keV of Au-NPs					
W40L	2.3	8.6	9.8	10.4	11.4	13.4
WMLA	2.4	8.6	9.8	10.4	11.4	13.4
WMDA	2.4	8.6	9.8	10.4	11.6	13.4
CMLA	2.2	8.6	9.8		11.6	13.8

From this study, the peak at 9.8keV was dominating at both waste water and municipal water. The municipal waste water samples with 40nm Au-NPs in light conditions, a mixture of 20nm and 40nm Au-NPs in light and agitation and waste water with a mixture of 20nm and 40nm in dark and agitation, all presented an Au peak at 10.4keV which does not appear in municipal water with a mixture of 20nm and 40nm Au-NPs in light and agitation. This peak (10.4keV) in waste water samples may be attributed to the presence of microorganisms in waste water that are responsible for synthesizing Au⁰ (gold). For example *E. coli* DH5 α , can synthesize gold nanoparticles (25 \pm 8nm) with mostly spherical shapes (Iravani, 2014). It was noted that sulphur peaks were not displayed on the EDX graphs even though it was the most abundant non-metal in the waste water.

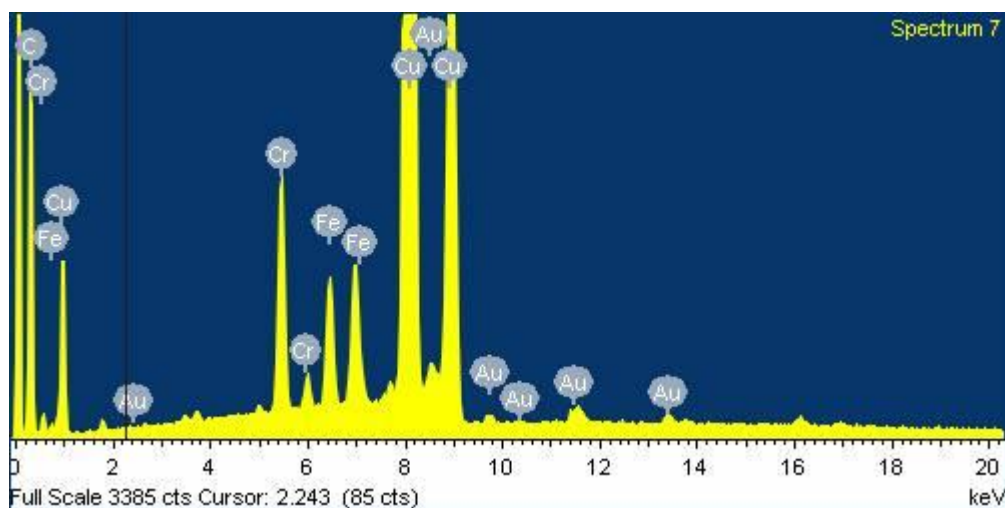


Figure 23: EDX micrograph for waste water with 40nm Au-NPs kept in light for one month (W40L)

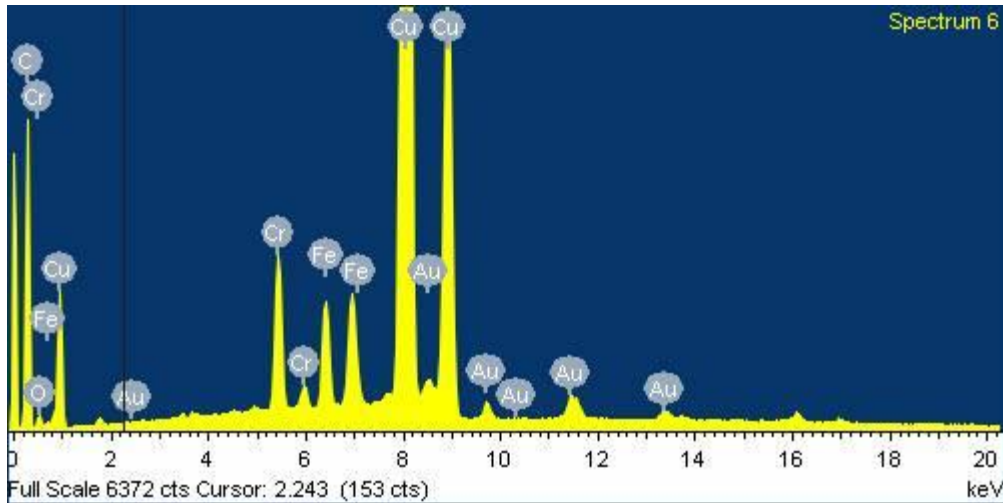


Figure 24: EDX micrograph for waste water with a mixture of 20nm and 40nm Au-NPs kept in light and agitation for one month (WMLA)

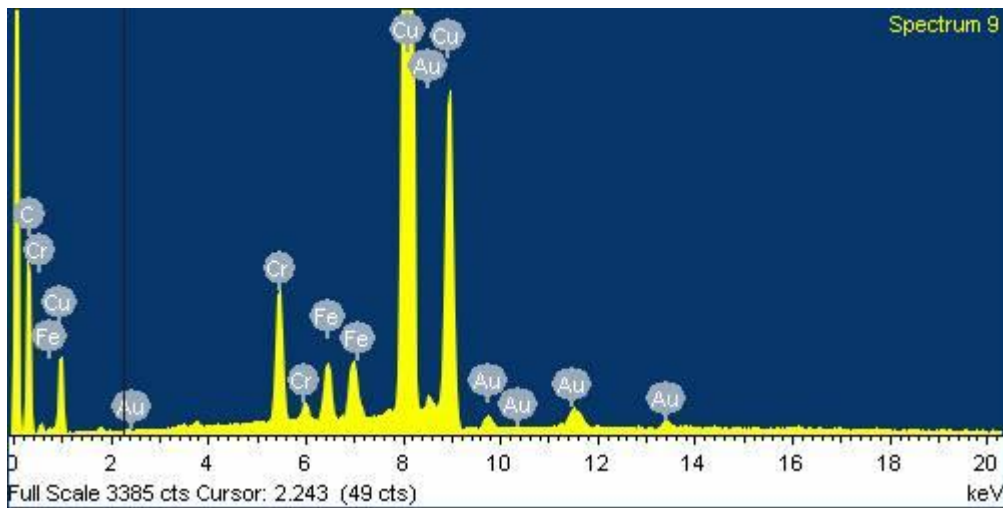


Figure 25: EDX micrograph for waste water with a mixture of 20nm and 40nm Au-NPs kept in dark and agitation for one month (WMDA)

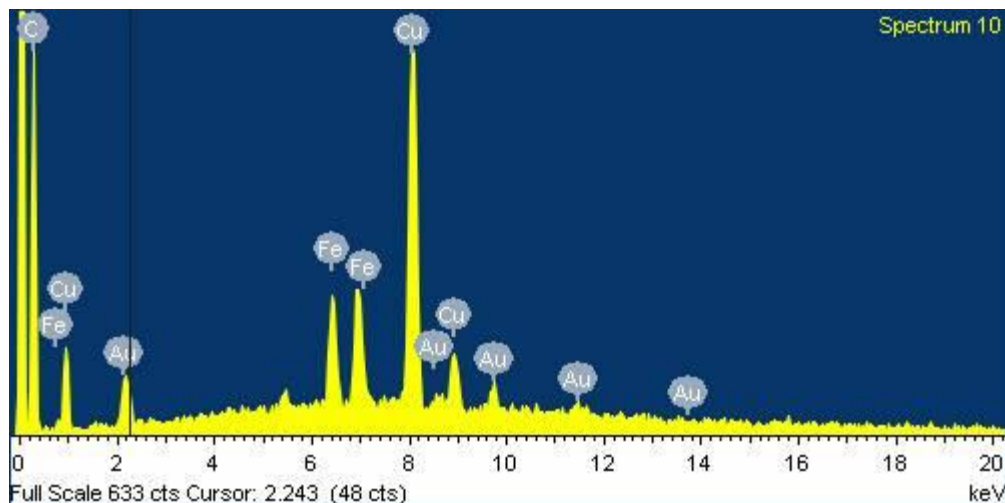


Figure 26: EDX micrograph for municipal water with a mixture of 20nm and 40nm Au-NPs kept in dark and agitation for one month (CMLA)

In a study done for the synthesis of gold and silver nanoparticles from *Trianthema decandra* root extract, Au atoms formed signalled at around 0.30, 2, 2.2, 2.4 and 9.7 keV (Geethalakshmi and Sarada, 2012).

7.4 Conclusion

The energy dispersive x-ray spectrometer (EDAX) showed the presence of elements such as C, Cr, Fe and Au. The elemental Au⁰ peaks at (2.2 -2.4), 8.6, 9.8, (11.4 – 11.6) and (13.4 – 13.8) were dominating at both waste water and municipal water indicating the presence of Au⁰ in the NanoGold particles. The presence of Au⁰ peak at 10.4 keV which did not appear in municipal drinking water with a mixture of 20nm and 40nm Au-NPs in light and agitation might present the presence of Au⁰ structure synthesized (or converted from one form to the other) by microorganisms in the waste water. The EDAX showed the existence of the disposed Au⁰ in either the municipal drinking water or the waste water for the duration of the experiment. This study showed that the disposed Au-NPs would exist in water and might probably be discharged into the water bodies.

Chapter 8: Conclusion and recommendations

8.1 Conclusion

The study assessed the morphological changes of Au-NPs in municipal drinking water (aerobic, light conditions) and waste water (anaerobic, dark conditions). The pH plays a very important role in the growth of microorganism and reactions that occur in the waste water. Fluctuations of pH in the study did not have any significant impact as it was still within the acceptable limits (5.5 - 9.5) with regard to modifying the environmental conditions of the waste water. Municipal waste water and drinking water with and without Au-NPs under aerobic conditions showed an overall increase in the COD levels while there was no significant change in COD in the waste water and municipal water under the anaerobic conditions. Addition of Au-NPs in waste water did not stimulate the variation (according to this study) of COD in the waste water; oxygen availability did have an impact as demonstrated by this study. Zeta potential of Au-NPs in municipal waste water in light and agitation showed that they were stable particles in the first month, but lost their stability on the second month. All other environmental conditions showed unstable Au-NPs in the two months period of the experiment.

The hydrodynamic size of Au-NPs in almost all municipal waste water samples was around 500d.nm or above during the first month of mixing. This might probably suggest some possible tendency of Au-NPs aggregation just after adding them in the municipal waste water. Otherwise it could be because of the sedimentation of Au-NPs on the wastewater sediment as the test samples were done from the sediment. The TEM results in comparison with their respective DLS results showed nothing of significance as some of the TEM contradicts their relative DLS. Generally there was no significant possibility of aggregation of Au-NPs in the municipal waste water. The EDX method revealed the presence of six gold peaks on municipal waste water and five of drinking water corresponding to Au⁰. This may be attributed to the presence of microorganisms in waste water that are responsible for synthesizing Au⁰ (gold). For example *E. coli* DH5 α , can synthesize gold nanoparticles (25 \pm 8 nm) with mostly spherical shapes thus adding more gold content in the waste water.

The study revealed that stable Au-NPs from the consumer nanoproducts can be rendered unstable along the waste water system after use. The instability can be unpredictable as they can react with other waste water constituents and produce unforeseen substances.

Disposing Au-NPs into the waste water system did not seem to have changed the environmental conditions of the waste water. Even though an intensive conclusion cannot be taken, considering the small amount of Au-NPs used in the study, much can be desired to be seen when large amounts of Au-NPs are disposed into the waste water systems

8.2 Recommendations

The use of nanoproducts with embedded nanoparticles (gold nanoparticles in this study) is rapidly growing with more and more particles accumulating in waste water in the near future. Even though this study did not prove beyond reasonable doubt the impact of gold nanoparticles abundance in waste water, may be because of the minimal amounts of gold nanoparticles used, there will probably be an impact when large amounts of nanoparticles accumulate in waste water. The loss of stability of gold nanoparticles in the waste water should be a cause of concern as they (Au-NPs) may become more reactive and could form unforeseen substances. Au is one of the valuable commodities, noting its possible presence in the waste water effluent, recycling could also be vital. There is a need for more research in this topic with larger amounts of gold nanoparticles with the inclusion of other mostly used nanoparticles like silver nanoparticles.

References

- Abdelhalim, M. A. K., Siddiqi, N. J., Alhomida, A. S., & AlAayed, M. S. (2012). Size effect of gold nanoparticles on various trace elements levels in different tissues of rats. *African Journal of Microbiology Research*, 6(10), 2246-2251
- Alkilany, A. M., & Murphy, C. J. (2010). Toxicity and cellular uptake of gold nanoparticles: what we have learned so far?. *Journal of nanoparticle research*, 12(7), 2313-2333.
- Auffan, M., Rose, J., Bottero, J. Y., Lowry, G. V., Jolivet, J. P., & Wiesner, M. R. (2009). Towards a definition of inorganic nanoparticles from an environmental, health and safety perspective. *Nature nanotechnology*, 4(10), 634-641.
- Bhattacharjee, B., Chatterjee, N., & Lu, C. H. (2013). Harmful impact of ZnS nanoparticles
Brar SK, Verma M, Tyagi RD, Surampalli RY. 2010. Engineered nanoparticles in wastewater and wastewater sludge – Evidence and impacts.US Environmental Protection Agency. *Waste Management* 30:504–520
- Chowdhury, S. (2013). Trihalomethanes in drinking water: Effect of natural organic matter distribution. *Water SA*, 39(1), 1-7.
- De Mes, T. Z. D., Stams, A. J. M., Reith, J. H., & Zeeman, G. (2003). Methane production by anaerobic digestion of wastewater and solid wastes. *Bio-methane & Bio-hydrogen*.
- DWA, (2009). Guideline document: package plants for the treatment of domestic wastewater https://www.dwa.gov.za/Dir_WQM/docs/GuidelineDocWEB.pdf(date accessed 21/06/2015)
- DWA (2013). Monitoring requirements (SANS 241:2011) and Drinking water quality compliance (2014 Blue drop limits) https://www.dwa.gov.za/dir_ws/dwqr/subscr/ViewComDoc.asp?Docid=594 (date accessed 21/06/2015)
- Elsaesser, A., & Howard, C. V. (2012). Toxicology of nanoparticles. *Advanced drug delivery reviews*, 64(2), 129-137.
- Fitch K and Kemker C. (2014). Algae, Phytoplankton and Chlorophyll. *Fundamentals of Environmental Measurements*. Fondriest Environmental, Inc. 22 Oct. 2014. Web.< http://www.fondriest.com/environmental_measurements/parameters/water_quality/algae_phytoplankton_and_chlorophyll/(date accessed 21/06/2015)
- García, A., Delgado L, Tora JA, Casals E, Gonzalez E, Puntos V, Font X, Carrera J, Sanchez A. (2012). Effect of cerium dioxide, titanium dioxide, silver, and gold nanoparticles on the activity of microbial communities intended in wastewater treatment. *Journal of hazardous materials*, 199, 64-72.

Geethalakshmi, R., & Sarada, D. V. L. (2012). Gold and silver nanoparticles from *Trianthema decandra*: synthesis, characterization, and antimicrobial properties. *International journal of nanomedicine*, 7, 5375.

Gericke, M., & Pinches, A. (2006). Microbial production of gold nanoparticles. *Gold bulletin*, 39(1), 22-28.

Glenn, J. B., White, S. A., & Klaine, S. J. (2012). Interactions of gold nanoparticles with freshwater aquatic macrophytes are size and species dependent. *Environmental Toxicology and Chemistry*, 31(1), 194-201.

González-Anaya, J. A., Nava-Alonso, F., & Pecina-Treviño, E. T. (2011). Use of Ozone for Gold Extraction from a Highly Refractory Concentrate. *Ozone: Science & Engineering*, 33(1), 42-49.

Hafner, B. (2006). Energy Dispersive Spectroscopy on the SEM: A Primer. *Characterization Facility, University of Minnesota*, 1-26.

Handy, R. D., & Shaw, B. J. (2007). Toxic effects of nanoparticles and nanomaterials: implications for public health, risk assessment and the public perception of nanotechnology. *Health, Risk & Society*, 9(2), 125-144.

He, S., Guo, Z., Zhang, Y., Zhang, S., Wang, J., & Gu, N. (2007). Biosynthesis of gold nanoparticles using the bacteria *Rhodopseudomonas capsulata*. *Materials Letters*, 61(18), 3984-3987.

Hitchman, A., Smith, G. H. S., Ju-Nam, Y., Sterling, M., & Lead, J. R. (2013). The effect of environmentally relevant conditions on PVP stabilised gold nanoparticles. *Chemosphere*, 90(2), 410-416.

Hotze, E. M., Phenrat, T., & Lowry, G. V. (2010). Nanoparticle aggregation: challenges to understanding transport and reactivity in the environment. *Journal of environmental quality*, 39(6), 1909-1924.

Hou, L., Li, K., Ding, Y., Li, Y., Chen, J., Wu, X., & Li, X. (2012). Removal of silver nanoparticles in simulated wastewater treatment processes and its impact on COD and NH₄ reduction. *Chemosphere*, 87(3), 248-252.

Iravani, S. (2014). Bacteria in nanoparticle synthesis: Current status and future prospects. *International Scholarly Research Notices*, 2014. <http://dx.doi.org/10.1155/2014/359316> [accessed 2/8/2015].

Jans, H., Liu, X., Austin, L., Maes, G., & Huo, Q. (2009). Dynamic light scattering as a powerful tool for gold nanoparticle bioconjugation and biomolecular binding studies. *Analytical chemistry*, 81(22), 9425-9432.

Kaegi, R., Voegelin, A., Sinnet, B., Zuleeg, S., Hagendorfer, H., Burkhardt, M., & Siegrist, H. (2011). Behavior of metallic silver nanoparticles in a pilot wastewater treatment plant. *Environmental science & technology*, 45(9), 3902-3908.

Karn, B and Aguar, P., (2007). Nanotechnology and Life cycle assessment proceedings of the workshop on Nanotechnology and Life Cycle Assessment. Washington DC

Kemker, C., (2013). pH of water. *Fundamentals of Environmental Measurements*. Fondriest Environmental, Inc. 19 Nov.2013. Web. http://www.fondriest.com/environmental/measurements/parameters/water_quality/ph/[accessed 2/8/2015].

Lapresta-Fernández, A., Fernández, A., & Blasco, J. (2012). Nanoecotoxicity effects of engineered silver and gold nanoparticles in aquatic organisms. *TrAC Trends in Analytical Chemistry*, 32, 40-59.

Lim, J., Yeap, S. P., Che, H. X., & Low, S. C. (2013). Characterization of magnetic nanoparticle by dynamic light scattering. *Nanoscale research letters*, 8(1), 1-14.

Madu, A. N., Njoku, P. C., Iwuoha, G. N., & Agbasi, U. M. (2011). Synthesis and characterization of gold nanoparticles using 1-alkyl, 3-methyl imidazolium based ionic liquids. *International Journal of Physical Sciences*, 6(4), 635-640.

Malvern Instruments, M. (2012). Dynamic light scattering: an introduction in 30 minutes. *Technical Note Malvern, MRK656-01*, 1-8.

Michelle Ly, E.I.T, (2013). Gold nanoparticles interaction with cell membranes. Unpublished BSc degree, Worcester Polytechnic Institute

Millar, N. (2001). Biology statistics made simple using Excel. *School Science Review*, 83, 23-34.

Musee, N. (2010). Nanotechnology risk assessment from a waste management perspective: Are the current tools adequate?. *Human & experimental toxicology*, 0960327110384525.

NanoComposix

nanoComposix. Zeta Potential Analysis of Nanoparticles. <http://nanocomposix.com/pages/gold-nanoparticles-physical-properties> [accessed 2/8/2015]. In. SAN DIEGO, CA; 2012.

Ngwenya, N., Ncube, E. J., & Parsons, J. (2013). Recent advances in drinking water disinfection: successes and challenges. In *Reviews of environmental contamination and toxicology* (pp. 111-170). Springer New York.

Odjadjare, E. E., Obi, L. C., & Okoh, A. I. (2010). Municipal Wastewater Effluents as a Source of Listerial Pathogens in the Aquatic Milieu of the Eastern Cape Province of South Africa: A Concern of Public Health Importance. *Int. J. Environ. Res. Public Health*, 7, 2376-2394.

- Owens, B. M. (2006). The potential effects of pH and buffering capacity on dental erosion. *General dentistry*, 55(6), 527-531.
- Pescod, M. B. (1992). Wastewater treatment and use in agriculture. <http://bvspers.paho.org/bvsair/e/repindex/rep84/vleh/fulltext/acrobat/wastew.pdf> [accessed 2/8/2015]
- Rastogi, I. D. (2012). Nanotechnology: safety paradigms. *Journal of Toxicol Environ Health*, 4(1), 1-12.
- Ray, PC., Yu, H., Fu, PP., (2008). Toxicity and Environmental Risks of Nanomaterials: Challenges and future needs. *Journal of Environmental Science and Health Part C*, 27(2009):1–35.
- Roux, S. P. (2008). Nanopollutants, their effects on the environment, and potential impacts on the South African water treatment and supply infrastructure.
- Schmidt, K. F. (2007). *Nanofrontiers: Visions for the future of Nanotechnology*. Project on Emerging Nanotechnologies.
- Schutte, C. F., & Focke, W. (2007). *Evaluation of nanotechnology for application in water and wastewater treatment and related aspects in south Africa*. Water Research Commission.
- Shon, H. K., Vigneswaran, S., Kandasamy, J., & Cho, J. (2007). Characteristics of Effluent Organic Matter in Wastewater. *Faculty of Engineering, University of Technology, Sydney, Australia*, 52-101.
- Sillanpää, M. (2014). *Natural Organic Matter in Water: Characterization and Treatment Methods*. Butterworth-Heinemann.
- Stankus, D. P., Lohse, S. E., Hutchison, J. E., & Nason, J. A. (2010). Interactions between natural organic matter and gold nanoparticles stabilized with different organic capping agents. *Environmental science & technology*, 45(8), 3238-3244.
- Tiwari, D. K., Behari, J., & Sen, P. (2008). Application of Nanoparticles in Waste Water Treatment 1.
- Trudel, S. (2011). Unexpected magnetism in gold nanostructures: making gold even more attractive. *Gold Bulletin*, 44(1), 3-13.
- USEPA, U. (2007). Nanotechnology White Paper. *Prepared for the US Environmental Protection Agency by Members of the Nanotechnology Workgroup, a Group of Epa's Science Policy Council Science Policy Council, US Environmental Protection Agency, Washington, Dc*, 20460.

Van Aardt, W. J., & Booysen, J. (2004). Water hardness and the effects of Cd on oxygen consumption, plasma chlorides and bioaccumulation in *Tilapia sparrmanii*. *Water SA*, 30(1), 57-64.

Vance, M. E., Kuiken, T., Vejerano, E. P., McGinnis, S. P., Hochella Jr, M. F., Rejeski, D., & Hull, M. S. (2015). Nanotechnology in the real world: Redeveloping the nanomaterial consumer products inventory. *Beilstein journal of nanotechnology*, 6(1), 1769-1780.

Wen, Q., Tutuka, C., Keegan, A., & Jin, B. (2009). Fate of pathogenic microorganisms and indicators in secondary activated sludge wastewater treatment plants. *Journal of environmental management*, 90(3), 1442-1447.

WHO, (2003). pH in drinking water.

http://www.who.int/water_sanitation_health/dwq/chemicals/en/ph.pdf (date assessed 21/01/2016)

WHO, (2011). Guidelines for drinking-water quality. *World Health Organization*.

Wilson, S. C., Duarte-Davidson, R. Z., & Jones, K. C. (1996). Screening the environmental fate of organic contaminants in sewage sludges applied to agricultural soils: 1. The potential for downward movement to groundwaters. *Science of the total environment*, 185(1), 45-57

Zhang, Y., Yang, M., Portney, N. G., Cui, D., Budak, G., Ozbay, E., & Ozkan, C. S. (2008). Zeta potential: a surface electrical characteristic to probe the interaction of nanoparticles with normal and cancer human breast epithelial cells. *Biomedical microdevices*, 10(2), 321-328.

Appendix

Table 4: The initial pH of the water samples and gold nanoparticles before mixing

	Range	Mean
Municipal water	6.82 – 6.83	6.83 ± 0.01
Municipal wastewater	7.13 – 7.15	7.14 ± 0.01
20nm Au-NPs	5.13 – 5.25	5.17 ± 0.07
40nm Au-NPs	3.80 – 3.82	3.81 ± 0.01