

**EMPIRICAL INVESTIGATION OF WATER POLLUTION
CONTROL THROUGH USE OF *Phragmites australis***

**A thesis submitted for the degree of
Doctor of Philosophy**

by

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Abstract

This research study addresses a problem of water pollution caused by heavy and toxic metals Cd, Cr, Cu and Pb. The thesis proposes the use of the technique of phytoremediation using *Phragmites australis* (PA) plants that have the capacity to absorb and to accumulate such metals in their roots and leaves. The metal uptake and their location of accumulation in the PA plants were determined using flame atomic absorption spectroscopy (FAAS) and transmission electron microscopy (TEM) respectively. Leachates from contaminated plant biomass were mixed with silver nitrate to assess the manufacture of metal nanoparticles as an added value step in the process from remediation to biomass disposal. Silver nanoparticles were readily manufactured by the leachates without, with the exception of copper, any incorporation of the pollutant metal. The presence of copper in the manufactured silver nanoparticles may be of some commercial use.

The results obtained show that PA plants will accumulate toxic metals when in hydroponic culture and that the majority of the accumulated metals are sequestered in the roots and do not enter the aerial parts of the plants in significant amounts. Silver nanoparticles were manufactured from the biomass using a low energy route with no additional chemicals, apart from silver nitrate thus reducing the environmental load that would otherwise be present if a chemical means of nanoparticle production was used.

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Declaration

The work described in this thesis has not been previously submitted for a degree in this or any other university, and unless stated otherwise it is the author's own work.

Statement of copyright

The copyright of this thesis rests with the author. No quotation from it should be published without prior written consent, and information derived from it should be acknowledged.

List of publications from this thesis

(1) Article published about nanoparticles manufacturing

Reynolds, A. J., Al-Akeel, K. A. and Verma, N., 2010, Comparison of the ability of a C3 and a C4 plant extract to manufacture silver nanoparticles.
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(2) Accepted Book Chapter

With Reynolds, A. J., Chaudhary, A. J., 2011, Copper uptake by *Phragmites australis* in hydroponic culture and the manufacture of silver nanoparticles from the biomass, in Bernd Markert (ed) '*Progress on heavy metals in the environment*', Leiden: Maralte Books

Glossary

Flame atomic absorption spectroscopy (FAAS): is a spectro analytical technique to determine quantity of chemical elements using absorption of optical radiation by free atoms in their gaseous state.

Heavy metals: This is a group or set of metals categorised based on their properties such as density, atomic weight, chemical properties or toxicity.

Microwave digestion: This is a method to make solution ready for leaching using the microwave.

Nanoparticles (NPs): Particles with size of 1 to 10,000 nanometers are classified as ultrafine, fine or coarse nanoparticles.

Oxidation state: Charge on an atom in a compound is its oxidation state. It is the number of electrons that an atom loses, gains, or appears to use when combining with another atom in compounds. It also determines the ability of an atom to oxidize (to lose electrons) or to reduce (to gain electrons) other atoms or species. Almost all of the transition metals have multiple potential oxidation states. Oxidation state of free elements is zero.

Phragmites Australis (PA) plants: is a commonly and naturally available plant in all habitats over the world. It is known for its ability to extract and to accumulate heavy and toxic metals in its stem, leaf and roots. It is also called reed grass or reed plant.

Phytoremediation: This is a technique to remove pollution from water or soil using absorption of toxic metals by microorganisms or plants such as PhragmitesAustralis (PA plants).

Rhizofiltration: This is also a technique to remove water pollution or accumulated nutrients through bioremediation by filtering water through plant roots.

Transmission electron microscopy (TEM): is a microscopy technique which uses a beam of electrons transmitting through an ultra thin specimen and interacting with the specimen as it passes through. The result is obtained using an imaging device.

Toxicity: This is a state of an element such metal which can kill any organism beyond certain level of quantity.

Xenobiotic: Xenobiotic is a pollutant not normally existing in organisms or expected to be present in them.

Ultra violet visible spectroscopy (UV-Vis): This is used to monitor the quantitative formation of nanoparticles of metals in the PA plants solution.

Energy dispersive X-ray analysis (EDX): To determine the location of metals.

Chapter 1 Introduction

1.1 Context of the thesis

This chapter of the thesis serves as a foundation for understanding the purpose, significance and structure of the research plan for the remainder of the project. Water, soil, air and crop pollution are the greatest challenges faced by human kind today. The removal of pollutants from water and soil has been long researched in the fields of environmental science and engineering to find ways of eliminating this serious problem in both the developed and developing world. Many different extraction methods have been developed empirically for commercial usage.

With the advent of technology, processes have become faster but bio, radioactive and toxic waste pollution have been increased to a level which can make resources like water, soil or plants hazardous when accumulated beyond a certain quantity. This research thesis addresses water pollution through heavy and toxic metals in detail and applies prevailing scientific knowledge and the laboratory experimentation to assess the abilities of *Phragmites australis* to extract soluble and toxic heavy metals from water. It also addresses the possibility to use the polluted biomass to add value to this material as a means of offsetting the costs of disposal.

The main context of this thesis is the use of PA plant with their ability to extract toxic metals through stem, leaf and root whilst growing. Combining the use of PA plants (or

Reed plants or Common reed) with cost effective and clean technology of phytoremediation researchers have developed an array of techniques to remove heavy and toxic metals from soil and water strata (Lee and Scholz, 2007; Jadia and Fulekar, 2009; Lu et al, 2010; Bianchi et al, 2011; Chen et al, 2012).

The central theme of this research project is to test 'reed plants' ability for waste water pollution control through use of the 'Phytoremediation' concept. '*Phragmites australis*' is a reed grass having important characteristics to absorb heavy metals and other toxic materials while growing in the polluted water. Thus, location and accumulation of heavy metals absorption is analysed in this study. These reed plants (also called PA plants) are commonly available in different habitat regions over the globe ranging from deserts and coasts to mountains. The plant itself also poses a problem when accumulated in fertile farming land and causes water leaching. Hence, this research uses various laboratory trials with solutions made with one or combinations of heavy metals such as Pb, Zn, Cd, Cu and Cr. The experiments also aim to investigate a novel use for the spent reed material after extraction of the toxic metals from water.

Nanoparticles are widely used in engineering, technology and health science and materials development. The post-production nanoparticles phase provides remaining material as bio-mass which has many other applications. Therefore, the main proposition of this thesis is to test an application of reed plants for pollution control based on their phytoremediation characteristics to absorb heavy metals as polluted waters. The other major issues in this study are measurement of heavy metals

absorbed by PA plants, location of heavy metals within the PA plants and the manufacture of nanoparticles from the biomass. The next section details the overview of the research process from existing academic literature.

1.2 Research overview

Environmental science provides the scope of studying various components of chemical processes related to water, soil and plants. This branch of science when interlinked with engineering applications, produce useful techniques and processes that can lead researchers to new manufacturing and control techniques for example, pollution control systems or nanoparticle applications. This research is divided into segments of heavy metal pollution, reed plant characteristics, pollution removal methods, laboratory methods and nanoparticle manufacture.

1.2.1 Pollution by heavy metals

Pollution from human made sources creates the conditions wherein metal presence is elevated to toxic levels in natural water resources. The major causes of water pollution include sewage, waste water, industrial waste, marine dumping, radioactive waste, oil pollution, underground storage leakages, atmospheric deposition and global warming. Now, water pollution has become a major problem globally and a leading cause of diseases worldwide with approximately 14000 people daily losing life because of water pollution (Pink, 2006; West, 2006). Both developing and industrialized countries are struggling to solve water pollution problems. Adding to this problem, industrial and technological developments have been using this important and natural source and made it a recipient of toxic, solid and liquid wastes from its variety of

usages, instead of preserving it. For example, national water quality report of USA states that 27.9% of assessed rivers and stream (miles), 42.9% of assessed lake, reservoirs and ponds (acres) and 37.2% of bays and estuarines (square miles) are classified as polluted (EPA,USA 2012). The preservation of aquatic resources for ecosystem and human health and wellbeing is a paramount concern worldwide and it has become evident that approaches to managing aquatic resources should be undertaken within the context of ecosystem dynamics in order that their exploitation for human utility remains sustainable (Nakamura et al, 2006). Exposure to toxic metals can cause serious health problems. For example, lead can be present in the tap water as a result of dissolution from lead pipes in the plumbing system, use of ceramic ware and food cans, dust or fume of paints, or from smelter processing of metal scrap, old batteries or cable sheathing. These are just few sources of lead contamination. There are other toxic metals apart from lead such as Cadmium, Mercury, Chromium and Arsenic which can cause severe health hazards when organisms are exposed to such metals.

The specific contaminants leading to pollution in water include a range of chemicals, pathogens, physical and sensory changes like high temperature and discolouration. Chemical contaminants may include organic and inorganic substances. Major research performed by Kamlbell et al (1996), MacDonald et al (1999), Boopathy (2000), Schreiber and Bahr (2002), McGuire et al (2005), Farhadian et al (2006) and Andreoni and Gianfreda (2007) in the field of soil contamination and bio-remediation of contaminated soil and water includes main points as listed below.

- Pollutant sources and concentration levels
- Chemistry and toxicity of contamination
- Solubility, transportation, adsorption, dispersion and volatility of pollutant compounds
- Detection, determination and monitoring of groundwater
- Chemistry and mechanics of soil at contaminated site
- Hydrogeology and hydrology of contaminated site
- Limitations of environmental standards for water and soil
- Environment conditions, nutrient sources and presence of electron acceptors

The term heavy metal refers for all the metals having density over 6. However, the official term toxic metal is more valid for the group of metals such as Cd, Cu, Cr, Pb, Hg and As. This is because some of them are essential within limits for human, animals, plants and other living organisms but in excess they prove to be toxic. The chronic exposure or quantity beyond permissible level makes them toxic for humans and other organisms. It is also valid for metals such as Zinc and Iron which are essential metals in humans. The term heavy metal has become interchangeable with toxic metal because usually toxic metals such as Cu, Cr, Cd and Pb all can be classified as heavy metal because of their density is in excess of 6g/cm^3 . For example, Cu – 8.93, Cr – 7.14, Cd – 8.65 and Pb has density of 11.34 g/cm^3 (Duffus, 2002). Also, these metals in their metallic state (valence 0) are not generally toxic unless they are fine enough to be breathed in or ingested. It is normally their various compounds that are toxic (Blaylock et al, 2000).

The toxic metals group (Cu, Cr, Cd, and Pb) is one of the major pollutants which contain elements with an atomic density greater than 6g/cm^3 . These elements' concentration in soil water varies from 1000 parts per million (ppm) to a few parts per billion (ppb) except Manganese (Mn). This Mn element is found in soil from 20 to 10,000 ppm. Soils described as metalliferous may have higher levels of certain elements. Beyond a certain level and environmental condition, these heavy metals in soil can cause pollution to living organisms and make the soil unusable for farming. Operations such as mining, energy production and agricultural activities using higher quantities of fertilizers have increased the concentration of these metallic elements in the soil causing hazardous pollution problems (Woolhouse et al, 1981; Alloway, 1995). This can lead to accumulation by living organisms (Sager, 1992) and levels of Cr, Cu and Zn reaching to toxic effects levels (Sager and Stoeppler, 1992; Ensley, 2000). The increased use of fertilizers contributes to the amount of metals in soils and water tables. The use of manure in developed countries has a higher rate of pollution through fertilizers; for instance, Copper is used to promote growth in animals especially pigs.

Eutrophication is the enrichment of surface waters with plant nutrients. It occurs naturally but normally it is a process of change from one trophic state to a higher trophic state by the addition or accumulation of nutrient(s). Agriculture is a major factor in Eutrophication of surface waters. Eutrophication is generally an increase in nitrogen and phosphorous causing accelerated plant growth, especially algae. The presence of toxic metals is secondary and is mainly from industrial sources.

Phosphorous and Nitrogen are mainly from fertilisers. When fertilizers are used extensively for higher yield all over the globe, they make the ground water table enriched with heavy metals from these fertilizers (Ongley, 1996). Therefore, fertilization of surface waters, both as a result of direct discharges of manure and as a consequence of nitrate, phosphate and potassium being leached from the soil causes contamination of the groundwater by toxic metals. High concentrations of these substances lead to acidification of water and pose a greater threat to the health of humans and animals. To a certain extent, these heavy metals keep accumulating in the soil as well and are extracted by crops which we consume for food. For example, acidification occurs as a result of ammonia emission (volatilization) from livestock accommodation, manure storage facilities and manure being spread on the land (Ongley, 1996). Therefore, controlled use of minerals and organic fertilizers along with sludge management is necessary to prevent water pollution through surface runoff and leaching of toxic metals to ground water tables. This can even lead to damaging DNA properties (Wang and Lin, 1995). The following table 1.1 shows the admissible and permissible level of these toxic metals in the fresh water. These standards are prepared and recommended by World Health Organisation.

Table 1.1 WHO standards for fresh water

Metal	Maximum Admissible Concentration mg/dm³	Maximum Permissible Level or Toxic level of metal mg/dm³
Cd	0.005	0.01
Cr	0.05	0.10
Cu	0.05	15.00
Pb	0.05	0.10
Zn	5.0	15.00

(Source: Bala et al, 2008)

From different sources of pollution, heavy metals are accumulated as high concentrations in water, soil sediments and plants. This poses a challenging problem to the world of science and human kind. Prevailing technologies to remove contamination from these type of polluted soil sites are isolation, mechanical separation, pyro-metallurgical separation, or chemical treatment these are efficient but expensive, labour intensive and soil disturbing (Mulligan et al, 2001).

More recently, the use of plants in metal extraction (phytoremediation) has been investigated as a potential alternative in the removal of heavy metal excess from soil and water (Chaney et al, 1997; Glass, 2000). This can be further categorised based on the contaminant or the mechanisms involved. Based on the contaminant, it can be degraded, extracted, contained or a combination of these; and as for mechanisms, phytoremediation can be classified as extraction, plant tissue concentrations, contaminant degradation, volatilization, immobilization at root level, and finally,

erosion level and infiltration control. Extraction processes when used in water based culture are called Rhizofiltration which is a maturing technology (Dushenkov et al, 1995; EPA, 2000). This can also be called phytofiltration which is based on hydroponically grown plants that have shown to be efficient in heavy metal removal from water. Plants such as *Eichhornia crassipes* (Mart) have shown potential to store 6000 ppm of Cd and Pb in the whole plant and more than 8000 ppm of Cu when grown with 5 ppm of these heavy metals (Sela et al, 1989, 1990). Another plant, the Water Fern (*Azolla filiculoides* Lam.) can extract and absorb elements like Cd, Cu, Ni and Zn. On individual exposure to these elements, *Azolla filiculoides* can uptake 10,000, 9000, 9000 and 6500 ppm of Cd, Cu, Ni and Zn respectively (Stratford et al, 1984). Aquatic plants such as *Nelum bonucifera* Gaertn and *Nymphaea alba* L. have been tested for their Cr uptake capacity. For example in experiments, these plants have shown accumulation of Cr to a level of 3000 mg Cr per kilogram of tissue. Cr is very toxic for most plants (Vajpayee et al, 2000).

As an example, Chromium (Cr) oxidation state has a range from (-II) to (+VI) with the most common oxidation states being (0), (III) or (VI); and Cr (III) trivalent is the most stable state. Naturally Cr is found present in complex cubic isomorphous minerals called Spinel. Cr can be melted with other metals to manufacture alloys or plating, for example chromium steel. In water, Cr concentrations are limited because of the low solubility of its oxides. Major chromium contamination is through waste water but no food or animal feed plants have been found with high chromium concentrations (Wang et al, 2009).

Hence, it can be inferred from the above discussion that water pollution can be resolved by using aquatic plants. In this scenario, governments, scientists and researchers face major problems: how to purify the polluted water and how to reduce pollution and keep it under control. The central theme of this research project is embedded in solving these two problems. Finding out the water purification method by means of empirical investigation using *Phragmites australis* (common reed plant) is the solution to this increasing problem. A permanent solution may be obtained by knowing what causes this pollution and using plants or other technology to prevent it.

Phragmites australis (PA) plant also known as common reed is a perennial plant which is widely used and has received considerable attention for remediating soils and water polluted by multi-metals due to its ability of thriving in various range of adverse conditions with rapid growth and high yields. Furthermore, reed plants can grow well in soils contaminated by Cd, Ni, As or wastewater contaminated with As. Previous long-term field experiments exhibited that giant reed is useful for eco-remediating soil contaminated by As, Cd, Pb and Zn (Papazogloua et al, 2005; Miao et al, 2012).

Government regulators, researchers and the public's concerns regarding ecological threats such as global warming and resource availability have increased leading to intensive research in new technologies such as removal of toxic metals using new economical plants based on existing and emerging remediation technologies (Sarma, 2011). Danh et al (2009) and Wang et al (2009) add that existing conventional methods of soil and water pollutant removal, such as chemical physical and microbiological methods are costly for installation and operation. Also, the pollution

problem is multiplied for industrialised, densely populated and developing countries due to increasing industries and population adding large amounts of toxic and hazardous waste into their environments (Zhuang et al, 2007; Wenzel, 2009; Bonanno and Giudice, 2010; Sarma, 2011). This has led to the evolution of plants based phytoremediation techniques and use of easily available reed plants for pollution removal.

The additional issue is removal of nutrients done by leaching, crop harvesting and water clogging. As a consequence of this, nutrient levels are increased in drainage canals. The aquatic grass is not useful in its primary role as an extra to but it can extract significant amounts of toxic and non-toxic pollutant materials (Wenzel, 2009; Lu et al, 2010). Phytoremediation is a process which uses plants for containment, degradation or extraction of Xenobiotics from water or soil substrates and it creates the opportunity of being used as an economic and non-destructive method to remove pollution from soils or water. This is confirmed by many researchers from laboratory experiments of plant tissues analyses performed on the leaves, stem and root samples for chlorophyll and mineral content (Raskin et al, 1997; and Shamsuddin, 2010).

Phytoremediation is the use of plants to remove, contain and render harmless environmental contaminants. This refers to all those biological, chemical or physical processes that can make use of plants to aid cleaning of contaminated substances. Two basic principles applied in this technique are phytoextraction (harvesting) and phytostabilization (root fixing) (Dushnekov et al, 1997; Raskin et al, 1997; Wang et al, 2009). Rhizofiltration is one category of phytoremediation technology which uses

plant roots to absorb, concentrate, and precipitate metals from wastewater which may include leachates from soil. Rhizofiltration makes use of terrestrial plants and not the aquatic plants because terrestrial plants have characteristics such as longer and fibrous roots covered with root hairs that have extremely large surface areas. The absorption of metals does not involve biological processes in the rhizofiltration (Salt, 1995; Kumar, 1995; Wang et al, 2009). In this study, phytoremediation is applied using the aquatic plant commonly known as reed plant.

The (Reed plants) aquatic plant characteristics of being able to extract and to accumulate metallic pollutants and also to keep growing at the same time is the basis of this empirical investigation (Zurayk et al, 2001; Bonanno and Giudice, 2010). These aquatic plants have two important characteristics of high metal absorption capacity and still be at the same time inactive in the water, which makes them major pollutant removers (Sawidis et al, 1995). Using these important findings, a process developed as Phytoremediation to extract xenobiotics from water and soil is an economic and non-invasive technique. Once polluting metals are absorbed from water using the above mentioned aquatic plants, the next issue of concern is the disposal of these plants containing the accumulated metals. Extracted heavy metals may be useful in the manufacture of metallic nanoparticles for industries (Tilman, 1996). So far, there has been little development in this line of research for author's country Saudi Arabia or elsewhere using grasses. Hence, this sequential procedure forms the basis of this experimental research. This thesis is centred on the basis of this concept of using plant technology for pollution control and removal. Present technologies such as filtration, absorption, chemical precipitation and ion-exchange are costly. However,

this proposed study of using reeds is more promising as compared to other methods. There are few empirical studies found in the literature wherein PA plants are exposed to multiple toxic metals such as Cd, Cr, Cu and Pb as reported in this study (Horsfall and Abia, 2003). Study would reveal novel findings about accumulation of each metal and behaviour of PA plants subjected to different toxic metals simultaneously.

1.2.2 Reed plants characteristics

The most common reed plant species, '*Phragmites australis*' (PA plants) can grow in natural or artificial conditions. It is found in North America, Europe, Middle East, Africa and Australia. It is generally found in low level coastal plains or river flood area but these plants also exist above sea level. PA plants can grow in height up to 3.5 metres and have ability to pass oxygen through its stems to roots. Roots can grow even in moist or water logged soil conditions.

The first ever use of PA plants is in the form of reed beds for water and sewage treatment which is the improved version of original contribution made by Prof. Kilkuth of Germany in 1970s. These reed beds utilize the plant's characteristics that it can transfer oxygen to roots and rhizomes which in turn increases the production of enzymes and that attracts bacteria to breakdown the pollution levels in the water or effluents. This method was first used in the UK in 1985 (www.constructedwetland.co.uk, 2009). Demirezen and Aksoy (2006) found that *Phragmites australis* is useful to measure concentration of heavy metals such as Iron and Manganese and PA plants can be used as biological indications in the determination of environmental pressures. Hence, literature and current industrial

applications suggest that *Phragmites australis* can be used in this study based on their characteristics as compared to other grass or plant species such as *P.pectinatus* or *G.densa*.

1.2.3 Pollution removal methods

Many metals are essential to life and living organisms' eco-systems. Low or excessive levels of metals can cause adverse effects on the health and the environment. The major toxic metals can be divided into two groups: 1. Lead, Arsenic, Cadmium, Mercury; and 2. Nickel, Zinc, Copper and Chromium. The first group Pb, As, Cd and Hg are non-essential (not required for normal growth) and the second group Ni, Zn, Cu and Cd are essential (necessarily required for normal growth) to plant and animals. These metals' easy transportation from one environment to another increases the complexity in controlling their measurement, accumulation level, removal or reduction. These metals can cause simultaneously air, water and soil pollution which has drawn much attention from researchers (Wang et al, 2009).

Heavy metals are considered as one of the biggest sources of environmental pollution due to their severe damage to human health and serious impacts on overall ecosystems (Roman-Silva et al, 2003). The sources of these toxic metals arise from industrial activities and inappropriate handling of effluents treatment processes. This requires understanding of each production method and each effluent treatment technique in addition to pollution removal methods. The sources of such toxic metal pollution are acid mine drainage, metal finishing and surface treatment operations. Leather tanning

processes, ferrous metal industries, coal-fired power generation, paint, pigments, plastic and food beverages also contribute a lot.

Industrial processes usually involve hazardous and pollution prone activities such as effluent from metal or uranium mines that carry sulphide, pyrite and pyrothite which are converted into sulphate in ground water streams. Uranium mining produces a large amount of radioactive material; electroplating during metal finishing generating toxic compounds of Cr, Cd, Ni, Cu, Fe, Zn and Sn for example organic pollutant EDTA, chromium tanning during leather tanning operations generate waste water with high proteins and sulfides containing dyes and sulfonated oils (Naja and Volesky, 2009).

The above mentioned pollutants are conventionally removed using various methods such as chemical precipitation, coagulation–flocculation, flotation, aeration, membrane filtration, ion exchange and electrochemical treatment (Philippis and Micheletti, 2009). Metal finishing industries mainly make use of sedimentation, electrolysis, ion exchange, evaporation, reverse osmosis and ultra-filtration (Vymazal et al., 2007; Shamma and Wang, 2009). Prevailing treatment methods for heavy metal pollution control are adsorption, sludge activation, phytoextraction, ion exchange, electro-kinetic and electro-osmosis processes (Ho and El-khaiary, 2009). These conventional methods are discussed in detail in the review of literature.

However, these methods are proving costly and resource consuming which have given way to new technologies such as phytoremediation using plants, grass and trees. The various new technologies that are used in the removal of these heavy metals from soil

and water are containment (immobilization), stabilization, vitrification, soil washing, soil flushing, pyrometallurgy, electro-kinetics and phytoremediation (Shammas, 2009). The aquatic macrophyte - *Phragmites australis* (PA) is one of the most common plants living in wet eco systems and is found all over the world. This plant can withstand extreme environmental conditions including toxic contaminants such as heavy or toxic metals (Quan et al, 2007). PA plants are widely deployed to construct wetlands to treat industrial waste waters containing metals (Bonanno and Giudice, 2010). The detailed analyses of these new methods are presented later in the thesis in literature review section.

1.2.4 Laboratory methods

The laboratory methods include main research application procedures and experiments required for this study. For example, ‘phytoremediation’ process to extract toxic metals from polluted water using reed plants is a research application. However, preparing a green house, using hydroponic culture whilst determining location, size and quantity of metal uptake are laboratory tests required to use such an application. Use of various apparatus such as hydroponic culture, microwave digestion, transmission electron microscopy (TEM), flame atomic absorption spectroscopy (FAAS) and ultra-violet visible spectrosopes (UV-Vis) are integral to laboratory experiments which are main phases of this research study. Two other important considerations are manufacturing nanoparticles and safe disposal of biomass, although the latter is not part of this thesis.

1.2.5 Nanoparticles manufacturing

The crucial aspect of removing polluted waste is how to dispose it off. Methods like incineration, landfills and aeration are common to non-metallic waste but when it comes to hazardous or heavy metals waste, one has to take extra care about their disposal. Nanotechnology advancement in the field of manufacturing technology has become first hand tool to create benefits from wastage metals (Watanabe, 1997).

Nanoparticles from natural sources have been present on the earth for millions of years for example due to volcanos and weathering. Now the technology to synthesize, combine and create nanoparticles has received much of the investment in terms of research and funds (Nowack, 2009). The ability of PA plants to extract different metal combinations and then produce NPs from biomass containing accumulated metals in the plant root and leaf is very significant in this study. This study involves manufacture of inorganic nanoparticles from biomass containing accumulated metals and to assess the effects, if any, on the composition and size of these nanoparticles (Nowack and Bucheli, 2007). Further discussion of complete laboratory methods and their behaviour will be explained in later chapters of this thesis.

1.3 Topic selection

As previously stated, water is one of the most important and scarce resource for communities spanning the entire world. However, the industrial and technological developments have been using this very significant source of our survival as a recipient of toxic, solid and liquid wastes from its variety of usages. These different sources of wastes get accumulated as high concentrations of polluting heavy metals in

the plants, sediments and other water resources. This poses the greatest challenge to the world of science, engineering and technology. These prevalent difficulties are faced by governments, scientists and researchers in many countries and Kingdom of Saudi Arabia is one of them. The author has studied agriculture science and has farming as his main profession. Thus, it was natural and obvious choice for him to select this topic for research to help solve a major problem for the country and research community.

1.4 Empirical proposition

Phragmites australis (PA) is a reed and a commonly found plant in the wetlands worldwide. Its habitat ranges from cool to arid regions and is a large biomass producer. It can be considered to be a pest in certain instances where it can slow down or create stagnation of running water courses. It is known as bio-accumulator or hyper-accumulator of certain heavy metals and therefore, has potential to be used as a phytoremediator of polluted water courses and soils through a green and economical process.

However, one can reclaim metal absorbed by PA plants and use plants as fertilizer since PA has the ability to regenerate after harvesting which would enable the sequential removal of metals from the waterways over several growing seasons. This harvesting will also free – up the water flow within the channels reducing stagnation. But this stage of reusing PA plants is not in the scope of this research study.

The proposed research involves laboratory growth under controlled conditions in the presence of single and multiple potentially toxic metal concentrations to assess the accumulation rates and inhibition / competition scenarios. This work will also involve use of various techniques to determine the location and quantity of metal uptake in PA plants, manufacture and size of nanoparticles. The following diagram summarises the complete theoretical and experimental theme of this project as a step by step process. This research will investigate the potential for reed plants (PA) to facilitate the remediation of metal polluted waterways in Kingdom of Saudi Arabia and to use the biomass generated for industrial purposes such as manufacturing nanoparticles.

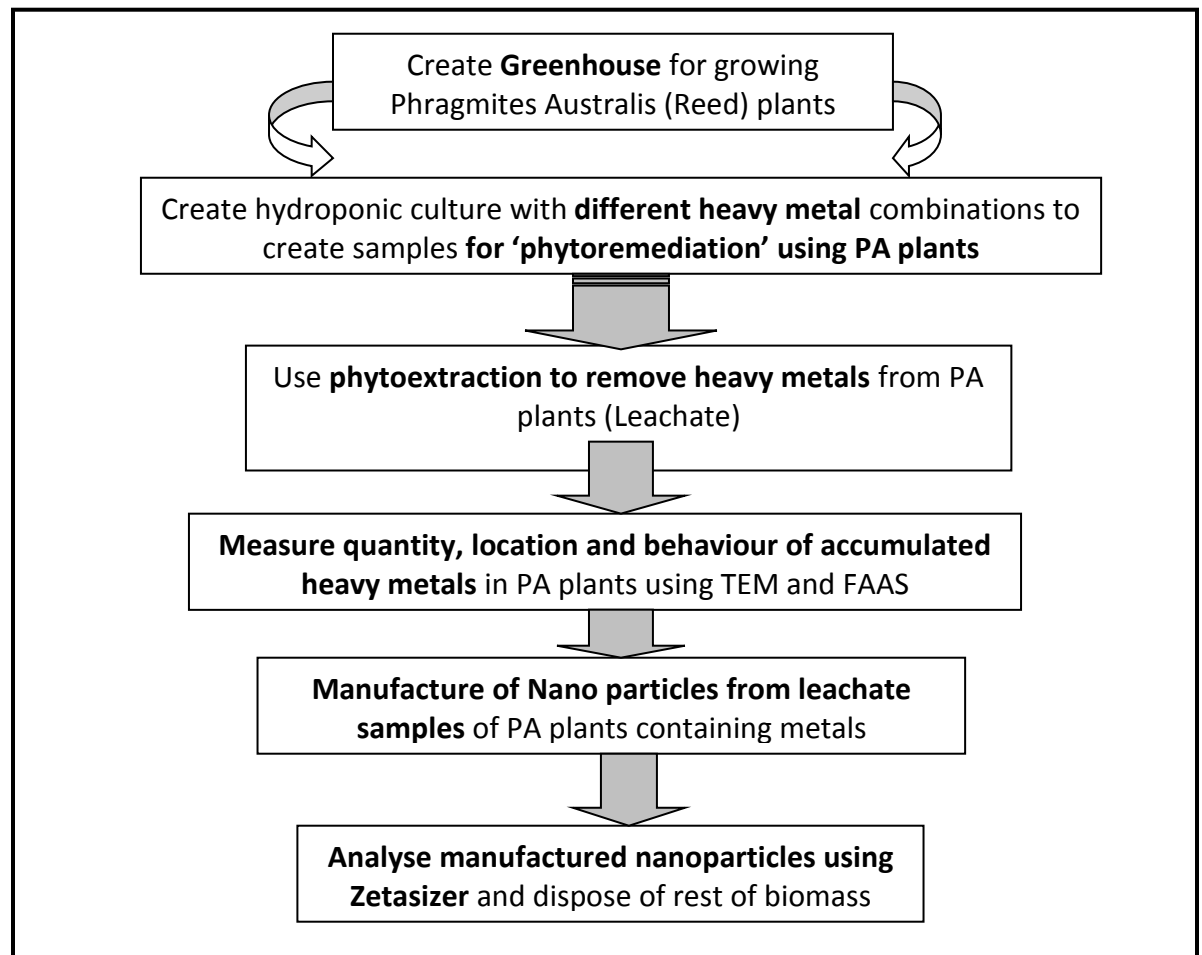


Figure 1.1 Empirical Research Plan

1.5 Aim and objectives

The main aims of this research study are to assess the capability of *Phragmites australis* a 'phytoremediator' to remove (or to decrease) metal pollution in water and to find a useful function for the biomass generated. In this process the author would fulfil the objectives mentioned below.

- a. Heavy metals absorptions and analysis techniques
- b. Nanoparticles formation and their further industrial use
- c. Biomass formation from reed plants, their use and disposal methods

1.6 Significance of the study

The purpose of this research is to benefit Saudi Arabia in its efforts to contain and reduce metal pollution and this will also benefit other countries with similar climates and pollution problems and the findings may also be applied to wider geographical areas. This research when finished with proven results can have further commercial applications and international implications, potentially leading to research publications and patents and surely, can benefit the environment around all of us in a time to come.

1.7 Thesis structure

The thesis is divided into seven sections, each described as one chapter and follows in a logical manner. First chapter comprises of back ground and overview information with precise problem definition and contribution of the research to the academia and industry. Second chapter reviews relevant academic literature and critically analyse it to find the solution from this secondary data. Third chapter introduces the proposed empirical research in detail. Fourth chapter provides complete outline of how each laboratory test is set up and how testing is carried out including all instruments and apparatus deployed. Fifth and sixth chapter discuss the results, findings and their detailed analysis. Seventh and final chapter discusses implications for theory and practice and concludes with research limitations, further scope of research and contribution made to the body of the knowledge and industry. Appendices include experiment data, observations, graphs and photos along with publications made during the research study.

Chapter 2 Literature review

2.1 Introduction

This chapter is necessary as a first point for finding an answer to the research problem defined in the previous chapter. This chapter reviews relevant academic research literature published pertaining to the subject under investigation and provides key definitions which will be useful in understanding the rest of the thesis. The chapter is structured in the sequential form of causes of the pollution by heavy metals problem and its prevailing solutions. It discusses various sources and effects of heavy metals and their behaviour as pollutant; use of plants for removing metal pollution and disposal of by-products generated from using such solutions. The major emphasis of reviewing literature in this section is to find out about latest trends, necessary information to carry out further research and supporting evidence to proceed further for the experimental research. Analyses of the published material in such a subject provide the advantage of knowing the controlling and critical points in the research before actually commencing the laboratory experiments. Continuing this discussion, next section outlines definitions of key terms for this project.

2.2 The research trends in heavy metals pollution and removal

Many metals are essential to organisms and eco-systems in the right concentrations. However, chronic exposure to even low concentrations of some metals can cause severe health and environmental damage while some of them are poisonous such as

arsenic, lead and mercury. The problem with heavy metals is that they do not degrade to become, for instance, carbon dioxide like some organic pollutants but get accumulated and transported from one environment to the other, these add to the issues of their containment, treatment, removal and disposal (Nriagu, 1988, 1989; Long et al, 1995; Ho and El-Khaiary, 2009). The research community, governments and businesses have attempted to address the issues of heavy metal contamination in air, water, soil and human bodies. Regulations and technology have been developed so that monitoring and prediction of metals contamination can be known and appropriate measures are taken (WHO, 1987; EU, 1996; US EPA, 1998). Various laboratory and in-situ techniques have been developed for heavy metals removal such as adsorption (Babel and Kurniawan, 2003); sludge activation (Brown and Lester, 1979); electro-kinetic (Pamukcu and Wittle, 1992); electro-osmosis (Acar et al, 1995), ion exchange (Ma et al, 1994) and phytoextraction (Baker et al, 1994; Dushenkov et al, 1995; Ebbs et al, 1997; Lasat, 2002). This research study pertains to the non-traditional method of phytoremediating characteristics of aqueous plants and further phytoextraction methods. The next section discusses sources of these toxic heavy metals.

Researchers have even attempted real time experiments or constructed large wetlands with reed bed to remove pollution (Lee and Scholz, 2007; Jadia and Fulekar, 2009; Lu et al, 2010; Bianchi et al, 2011; Chen et al, 2012). These experiments include waste water treatment, sludge treatment, sewage water treatment, polluted industrial water treatment wherein PA plants, Switch grass or plant bed are exposed to multiple metals

polluted solutions or samples (Alkaorta et al, 2004; Tripathy et al, 2007; Guala et al, 2011; Miao et al, 2012).

2.3 Sources and behaviour of toxic heavy metals

Metals are found in the Earth's crust, ocean or in the pure state such as gold. To date, 80 metals are found in total of 109 identified elements in the periodic table. Metals are substances having the common characteristics of shine, heat and electrical conduction, malleability, ductility and hardness except alkali metals such as Li, Na, K, Rb, and Cs, which are soft (Csuros and Csuros, 2002,). Strong electrostatic attraction between the proton containing positive charges, nuclei, and negative electrons explain these characteristics (Csuros and Csuros, 2002). They are in solid form at room temperature except Mercury which is in its liquid form.

Most metals in their metallic or zero valence state are insoluble. Sodium is an exception which reacts with water. Some compounds of metals easily dissolve in water and some do not, for example lead carbonate which is insoluble. Since these metals are not degradable metabolically, their accumulation in living tissues can cause serious health effects. Some metal compounds can be dissolved in water which may result in introduction to soil and thus transfer to food chain. Many industrial activities such as mining, processing minerals and chemical industries, increase non-essential metal concentration in the water tables in the surrounding regions of their manufacturing plants (Ho and El-Khairi, 2009).

The oxidation of pyrite and sulphide minerals in presence of moisture forms acid-mine drainage water (Naja and Volesky, 2009). This acid-mine drainage water dissolves the toxic elements from tailing and soils to waterways and ground water tables (Naja et al, 2008). Mining operations deposit high levels of iron, manganese, lead, zinc, copper, arsenic and silver into drainage basins and other aquatic environments by the discharge of mining industrial effluents (Wang et al, 2009). Domestic waste water effluents contain the large quantities of trace metal elements from metabolic waste, detergents or pipe corrosion which produce high levels of copper, lead, zinc, cadmium, iron, manganese, chromium, nickel, cobalt, zinc, boron, arsenic and mercury (Curos and Curos, 2002). Other sources of high content of heavy metals are storm water runoff, industrial wastes and discharges, sanitary landfills, agricultural run off and fossil fuel combustion (Wang et al, 2009).

As shown in the periodic table 2.1, metals are classified into groups and sub-groups. For example, main or A group includes metals found in the earth crust as free metals such as gold, silver, copper, iron, lead, mercury and tin. This category has five sub-groups as shown in the table 2.2 which provides the classification of metals and their sub-groups based on their physical and chemical properties. In the periodic table, they are divided into three major segments of metals, non-metals and metalloids. For this study, the researcher has selected four heavy metals commonly found as pollutant in different habitats in water tables of Saudi Arabia.

The selection of four metals for this study Pb, Cr, Cu and Cd is highlighted in the following table:

Table 2.2 Classification of metals

Main Group Name	Sub-group name	Metals included in that sub-group
Representative metals A Group	Group IA 1 Alkali metals	Li, Na, K, Rb, Cs, Fr
	Group IIA 2 Alkaline earth metals	Be, Mg, Ca, Sr, Ba, Ra
	Group IIIA 3	Al, Ga, In, Tl
	Group IVA 4	Sn, Pb
	Group VA 5	Bi
Transition metals B Group		Sc, Ti, V, Cr , Mn, Fe, Co, Ni Zn, Y, Zr, Hf, Nb, Ta, Mo, W, Tc
	Coinage metals	Cu , Ag, Au
	Platinum metals	Ru, Os, Rh, Ir, Pd, Pt
	Free metals	Cd , Hg
	Inner transition elements	La, Ac
Metalloids	Group IVA	Si
	Group VA	As, Sb
Heavy Metals	Class A and Class B metals	Metals with density greater than 5g/cm^3 that is specific gravity greater than 5.
Metals with multiple Oxidation states		Cu , Hg, Fe, Cr , Mn, Co, Sn, Pb , Ti
Metals essential to life	Metals important to human metabolism	Ca, Fe, Cu , Na, K, Mg, Zn, Mn, Co, Cr (III), Se

(Source: Csuros and Csuros (2002), Wang et al (2009))

Pb, Cr, Cu and Cd are considered as heavy metals because they have characteristics of forming positive ions in the solution and each has a density more than 5 gm/cm^3 . Each of these metals is discussed below in terms of their properties, significance, and

toxicity and health effects due to exposure to them. Metalliferous soils can contain high amounts of heavy and toxic metals as shown in the table 2.3

Table 2.3 Heavy metals concentration

Concentration of heavy metals in soil and plants mgkg^{-1} (ppm) and clean up criteria

Element	Normal range in soil[1]	Critical soil concentration[1]	Normal range in plants[2]	NJDEP non residential clean up criteria[5]	Concentration in metalliferous soils[2]
Cd	0.01-2.0	3-8	0.1-3	100	11-317
Total Cr	5-1500	75-100	0.2-5	NA	47-8,450
Cu	2-250	60-125	5-25	600	52-50,900
Hg	0.01-0.5	0.3-5	0.19.5 ^a	NA	100-400 ^b
Ni	2-750	100	1-10	2400	19-11,260
Pb	2-300	100-400	01.-5	600	3,870-49,910
Zn	1-900	70-400	2-400	1500	109-70,480

(Source: Gardea-Torresday et al, 2005, p.1798)

Cadmium (Cd)

Cadmium is a silvery-white, lustrous, but tarnishable, soft, ductile metal and has a relatively high vapour pressure. Cd is divalent, chemically resembles Zinc and is found in many Zinc ores through isomorphous replacement (Naja and Volesky, 2009). This metal is available in less quantity than Zinc and is usually in the form of an impurity in the Zinc ores and considered as by product of Zinc mining. When it is in free condition, it is available as soft, malleable, ductile and moderately active similar to other stable metals Zinc and Mercury because of its oxidation state of +2 like Zinc and low melting point characteristic like Mercury (Csuros and Csuros, 2002). Cd is a

rare and uniformly distributed element in the Earth's crust with an average concentration of 0.15 to 0.2 mg/kg. It occurs in the form of inorganic compounds and complexes with chelating agents (Hiatt and Huff, 1975 as cited in Naja and Volesky, 2009). The solubility of Cd in water is affected by the acidity of the medium. The increasing acidity of the solution will lead to more dissolution of suspended or sediment bound cadmium. The concentration of Cd in unpolluted fresh water is usually found to be less than 0.001 mg/L and in seawater 0.00015 mg/L. The presence of Cd in vegetation might be due to absorption through roots or depositing Cd containing aerosols on the plant surfaces (Fleischer et al, 1974 as cited in Naja and Volesky, 2009).

Main sources of Cd pollution are industrial and municipal wastes. The main usage of Cd is in coating other metals as a protective layer and in nickel – cadmium, silver–cadmium or solar batteries, electroplating, paint pigments, plastics, transportation equipments, machinery and baking channels, photography, and television phosphors (Csuros and Csuros, 2002). The utility of Cd is growing at an annual rate of 5 to 10% and current Cd production in the world is 20,000 tonnes. Industries like battery manufacture and smelting operations can affect the environment at a distance of a 100 km of magnitude (Bernard and Lauwerys, 1984 as cited in Naja and Volesky, 2009).

Cd is not an essential nutrient for animals or humans. The toxicity of Cd is very high because, if Cd is absorbed even in the small quantity by the body, it can cause severe high blood pressure, heart disease and it can lead to even death (Pan et al, 2010). The acute over-exposure to Cd fumes can cause pulmonary diseases and chronic exposure

can cause renal tube damage and prostate cancer (Jarup and Akesson, 2009). Cigarette smoke has high level of Cd which causes local air pollution and water pollution is caused by small Cd from mining, industrial operations, leaching of landfills and galvanized pipes corrosion. Absorbed Cd is accumulated in the renal cortex and liver while pancreas, thyroid and gall-bladder can also have higher concentrations. Cadmium accumulation in the human body happens over the age up to 50 years when it reaches peak or maximum level. Cadmium, due to its high toxicity levels, has been considered one of the most toxic metals in line with lead and mercury (Nriagu and Pacyna, 1988). Cadmium can be easily and very strongly absorbed by living cells. The toxicity of Cadmium can be understood from these examples, doses as low as 15 to 30 mg from acidic food stored in Cd-lined containers have caused human subjects acute gastroenteritis. Fluid consumption to a level of 13 to 15 mg/L can have caused vomiting and gastrointestinal cramps. An average American male has total 30mg Cd in the body with 33% in kidney and 18% in liver. The most common disease is kidney stones in human body from chronic exposure to Cd (Nriagu, 1980).

Copper (Cu)

Cu a reddish brown, malleable, ductile, high conductor of electricity and corrosion resistant metal is usually found in the metal ores containing sulphides, arsenides, chlorides, and carbonates (Csuros and Csuros, 2002). Cu is mainly used in plumbing and electrical conductors. Cu oxidizes slowly in air and, in the presence of CO₂, its surface exhibits a green colour due to a film of Cu₂ (OH)₂CO₃ (Flemming and Trevors, 1989). It is usually exists in the +2 oxidation state. CuO – copper (II) oxide which is black, but can also exist in the +1 state Cu₂O – copper (I) oxide which is red.

Trace amounts of copper are essential to life because it plays a major role in the enzyme function as a co-enzyme, but in large amount is toxic (Abel, 2002).

Chromium (Cr)

This transition metal is found at a degree of oxidation from $-II$ to $+VI$. The most common oxidation levels of Cr are $+6$, $+3$ and $+2$. In nature, Cr is found in the form of complex cubic isomorphous minerals called Spinel. The most stable state of Cr is the $+3$ valence state which is common for its existence (Wang et al, 2009). Cr is a rare industrial metal which is grayish-white in colour, very hard and has a high resistance to corrosion. An invisible oxide coating on the surface of Cr helps it to maintain brightness. This property allows Cr to become a protective and a decorative coating for other metals such as brass, bronze and steel. Chrome plate is used in the automobile parts such as bumpers but Cr is applied electrolytically (Mohan et al, 2006). Stainless steel is an alloy of iron with typically containing 18% Cr, 8% Ni with or without small amounts of silicon and molybdenum. Chrome steel contains a higher concentration of chromium but no nickel, with possibly small amounts of manganese, carbon, phosphorus, sulphur and silicon depending on the alloy. Chrome steel alloys provide high corrosion resistance and good hardenability (Mohan et al, 2011). Other applications of Cr include tanning agents, paint pigments and catalysts to impregnation solution for wood or photography.

Cr^{+3} has not been found with any hazardous effects however, Cr^{+6} is counted as lethal for any dose higher than 3g for adult human body. Cr^{+6} with H_2SO_4 form a powerful oxidizing medium that is utilized as cleaning solutions. Cr^{+3} may act as nutrition to

human body but Cr^{+6} is highly toxic and intake of Cr^{+6} can cause haemorrhaging in the liver, kidneys and respiratory organs. Exposure or excessive inhalation of Cr^{+6} dust has shown the development of dermatitis, ulceration, gastric cancer and perforation of the nasal septum in workers (Mohan and Pittman, 2006). Cr concentration is found to be low in fresh water due to low solubility characteristic of Cr^{+3} oxides.

However, chronic and acute exposure to Cr^{+6} can lead to increasing lethal symptoms; for example, it may start with vomiting and persisting diarrhoea and may rapidly lead to hemorrhagic diathesis and epistaxis and then convulsions as a final stage of illness. It can also damage liver and kidney, and cause respiratory problems, irritation and ulceration (Mohan et al, 2011). In plants, severe toxic effects are observed even with 0.5 mg/L concentration (Wang et al, 2009; Mohan et al, 2011). Chromium is beneficial as well as toxic. Cr^{+3} is an essential trace element for metabolism, it is also used for reducing blood sugar levels to control diabetes. Cr^{+3} can be found from various foods such as cheese, whole grain breads, cereals and broccoli (Kimbrough et al, 1999). Industrial applications of chromium compounds which causes water pollution are electroplating, metal finishing, magnetic tapes, pigments, leather tanning, wood protection, chemical manufacturing, brass, electrical and electronic equipment, catalysis (Mohan et al, 2011).

Lead (Pb)

Lead is one of the most common heavy elements. Out of several stable isotopes, it is the most abundant. Lead occurs as Galena (PbS). Early use of lead was in water pipes and pipe joints. Pb is a very heavy, soft, highly malleable, bluish – grey colour metal

and contains two oxidation states, +2 and +4. Pb resists corrosion and has a low melting point of 327° C. Solid and liquid sludge wastes contribute more than half of the Pb contamination into environment, mainly through the landfills. The other major lead pollutant is exhaust fumes of cars which cause atmospheric pollution (Naja and Volesky, 2009).

Lead pollution is typical example of anthropogenic metal pollution. Over 2700 years, lead pollution increased during the industrial age and has risen rapidly since it was added to gasoline fuel of vehicles (Valavanidis and Vlachogianni, 2010). Worldwide lead production is approx. 3 million tonnes per year of which 40% is consumed by electrical accumulators and batteries, 20% in alkyl additives in gasoline, 12% in construction, 6% in cable coatings, 5% in ammunition and 17% in the miscellaneous usage. Approximately 20% of total mined lead is lost as wastage in manufacturing processes. Pb is used to make batteries, solders and gasoline octane boosters. However, due to environmental hazards many countries have stopped lead additive usage in gasoline (Naja and Volesky, 2009).

Lead due to its common use in plumbing can be found in tap water; and amount of Pb depends on factors such as pH, softness and standing time of water in the piping system. Pb is toxic to the human nervous system and children are more susceptible to its effects. Lead can go to the central nervous system directly from the intestinal tract by absorption (Abel, 2002; Naja and Volesky, 2009). Sources of Pb contamination are Pb containing paint, air, soil, dust, food and drinking water. Pb presence in the body can be measured in the blood levels as micrograms of Pb per decilitre of blood

(microgram/dl). More than 10 microgram/dl can cause severe health effects (Cicchella et al, 2008). Due to regulatory control on the use of lead in gasoline, paint and food cans, exposure to lead has declined from the 1980s. Pb can be absorbed by human subjects in the different modes such as inhaling, ingestion, dermal contact or through the placenta. Young children absorb more Pb from ingestion through food compared to adults through the habit of eating clay (pica). Once lead is absorbed in the soft tissues of blood, lung, liver, spleen, kidney or bone marrow, it gets rapidly spread as poison in the body at faster rate as compared in hard tissues of bones or skeleton where Pb is slow to spread. The biological half-life of Pb in blood is approx. 16 to 40 days and in bones about 17 to 27 years (EPA, 1990). Inorganic Pb is considered as a general poison and enzyme inhibitor whereas organic lead is more poisonous than inorganic Pb. The usual symptoms are psychological for example, excitement, depression and irritability. Young children can develop mental retardation and brain damage. The most insidious characteristic of Pb is the ability to replace the calcium in the bones and to form a semi-permanent reservoir for long term release after the initial absorption of Pb. This can be measured by blood tests and radiography. Due to the chronic exposure to lead through sources such as plumbing system or atmospheric fallout or soil pollution, the human body accumulates Pb. The intake and uptake of Pb into the human body from all sources usually reaches to the level of permitted threshold of toxicity (Baker et al, 1984). Thus, there is no margin left for increase in Pb exposure to the human body. Pb is a cumulative poison and foetuses, infants and children are more susceptible to adverse health effect of Pb. Pb can directly affect the central and peripheral nervous system resulting into multiple neurological and electro-physiological effects (Naja and Volesky, 2009).

2.4 Water pollution and prevalent treatment methods

Water is one of the most important resources for human life on the earth and is abundant on the earth but drinking water resources are constantly depleting over time. This is a serious concern for the global population, regulators and scientific researchers. This section discusses water pollution sources and prevalent methods to reduce pollutants from water (Liu et al, 2011; Mohan et al, 2011).

2.4.1 Water quality constituents

Temperature is a major factor in regulating many natural processes of water within the environment (hydrothermal cycle). Temperature affects physiological functions in organisms and its effects include the rate of chemical reactions, enzymatic functions, molecular exchanges and movements between membranes within systems and organs. Based on temperature changes in the environment, organisms or processes within organisms can be accelerated, slowed, restricted or terminated. Since temperature can substantially influence the metabolic rate, it is one of the most important factors in water quality. Dissolved oxygen is a major water quality component which is required for keeping organisms healthy, sustaining species reproduction, vigour and the development of populations. For example, if dissolved oxygen content in the water fall below 3 mg/litre then it can be a cause of reduced growth rate, reduced toxic tolerance and blood clotting (Mackenthun, 2005).

pH which is defined as a negative decimal logarithm of the hydrogen ion activity in a solution is another important physical characteristic of water. It can be expressed mathematically as an equation (Covington, et al, 1985),

$$\text{pH} = -\log_{10}(a_{\text{H}^+}) = \log_{10} \left(\frac{1}{a_{\text{H}^+}} \right)$$

The range of pH is 0 to 14 whilst 0 to 7 is considered as acidic and 7 to 14 as basic or alkaline. It is known that pH Between 5 to 9 is generally not lethal for most of the plants, however, more extreme values such as below 4 or more than 10, are undesirable and hazardous in waters. Drinking water with pH 7 is considered as neutral on the scale.

Water quality can be affected by light as rooted, suspended and floating aquatic plants need light for photosynthesis. Light penetration into water is usually affected by microscopic plants, animals, and suspended mineral particles, stains that impart a colour, detergent foams, floating or suspended debris. The stratum in which light is adequate for photosynthesis in plants is referred as trophogenic zone which is the layer that receives 99% of the incident light. The depth of Trophogenic zone may vary between less than 5 to more than 90 feet. Also, the depth of water and seasonal variation of the solar light intensity influence photosynthesis rate (Mackenthun, 2005).

The velocity of water movement or flow is very significant to aquatic life and organisms in the water as it affects the transportation of nutrients, organic food attached to stationary surfaces, transport of plankton and benthos, addition of oxygen through aeration. Silts which moved downstream are often the cause of increased

content of nitrogen and phosphorus. Microscopic materials and pollutants such as fibre and wool drift with the other sediments and are deposited downstream and thus, flow of water determines the species in the stream bed organisms (Abel, 2002).

Silt content in the water is a main cause of turbidity through decomposition of sediments which often destroys insect and other aquatic populations such as mussels. Sediments and increased turbidity create covers at the bottom of the water stream in the form of silt blankets. This reduces the water table available for living organisms such as fish and algae within the waterbeds (Abel, 2002).

Oil or its by-products suspended in the water or their coating on surface of water can destroy algae and plankton, can interfere with re-aeration and photosynthesis or can cause direct toxicity. Oil can severely damage marine species and other aquatic life by poisoning it with ingestion of oil or soluble fractions such as phenol, ammonia and sulphides (Fergusson, 1990; Abel, 2002; Wang et al, 2009).

Nutrients by natural means or anthropogenic sources can enrich the water resulting in Eutrophication. It has been found that phosphorous and nitrogen from fertilization; Iron, trace and other heavy metals from effluents and sewage are responsible for increase in eutrophication. In some cases eutrophication caused by nutrients, pure elements and metals is found to be beneficial for growth of aquatic life (Wang et al, 2009). For example, algae growth is influenced by vitamins, trace metals, hormones, auxins, extracellular metabolites, auto-intoxicants, viruses, predation and grazing by aquatic animals. However, eutrophication causes pollution when such elements

contents increase beyond their toxic level threshold. Thus, pollution and eutrophication of water create two problems: the first problem is human life can be damaged by any chronic or excessive exposure to these nutrients and toxic metals. The second problem is, once these toxic metals and nutrients are dissolved into the water, then their reduction or removal is complex, time consuming and costly (Rabalais et al, 2009). These may be either by controlling inflow of these toxic elements or their removal or outflow. Dredging is considered a usual tool for removing the stored nutrients from beds of sediments. This activity deepens an area where nutrient removal takes place and this increased depth in the lake bed can be advantageous if it can contain the growth of unwanted plants. Even if phosphorus is considered beneficial for plants growth, researchers suggest that to prevent toxic effects of phosphorus, its content should not exceed 100 µg / litre at any point in the flowing stream and not exceed 50 µg / litre in the standing (non-moving) waters (Abel, 2002).

Other toxic substances include many pesticides and heavy metals flowing into the waters from industrial waste waters, sewage water, surface drainage and crop fertilization. As discussed above, the toxicity of these substances depends upon the water quality characteristics – temperature, pH, turbidity and alkalinity (Mackenthun, 2005).

2.4.2 The scale of water pollution

Water pollution research and development of remedial techniques for water pollution has become the focus of public and governments concerns worldwide in the last three

decades. The surprising fact for UK is that more pollution is caused and distributed by agriculture rather than industry (Abel, 2002). The cause of pollution can be accidental, negligence or illegal discharge of polluted wastes to water sources. Such incidences have gained more attention now days because of strict regulations, monitoring of pollution sources and increased public awareness (www.environment-agency.gov.uk, 2011). These issues reflect as a cause of more pollution in KSA as compared to developed western countries. The experience of pollution control in developed countries has proved that developing economies like the Kingdom of Saudi Arabia (KSA) shall attempt to combine economic and industrial development whilst demands for water resources and pollution control increase (Mackenthun, 2005; Wang et al, 2009).

Pollution spreads easily to surface and underground water tables by industrial, and agricultural generated flow of toxic chemicals and metals into ponds, lakes and rivers. High concentrations of toxic metals can make water metalliferous and non-palatable. Pollution control requires mapping of water pollution distribution which can be carried out with investigation techniques of taxonomy, ecological sampling and chemical analyses. Metal pollution affects the invertebrate fauna of the rivers through elimination or reduction of organisms. If pollution input can be controlled, then the fauna and quality of water may be recovered with time. Marine organisms are usually able to survive if the level of toxicity is less but then continuous exposure to even sub-lethal toxicity eliminates these organisms. Thus, concentration and exposure time both are crucial factors in pollution control of waterways. This thesis takes into account these factors for hydroponic experiments using *Phragmites australis* (common reed)

plants. In this thesis, PA plant growth and metal uptake are observed through varying degree of metal concentrations, combinations and exposure time measured in weeks.

2.4.3 Sources and effects of water pollution

The major and the most common source of water pollution is the discharge of effluents from sewers, treatment plants, drains and factories; this type of pollution source is known as point source discharge. Water pollution from such a source decreases with a distance from point of discharge and is easy to monitor and to control. On the other hand, in diffuse source pollution, pollutants enter water from multiple sources such as atmosphere, surface drainage, ground water infiltration, plant nutrients from fertilisers and pesticides. The effects of diffuse source pollution can be severe as it is difficult to control. Most methods are developed to control point source pollution. This pollution depletes the oxygen quantity and affects the temperature and pH levels which must be balanced for survival of aquatic organisms in the environment (Abel, 2002). For example, oxygen is a limiting factor for fishes; with temperature increase, the fishes require more oxygen because of increased metabolism and at the same time solubility of oxygen decreases because of higher temperatures (Varley, 1967; Alexander, 1970). Thus, pollution can directly influence environmental equilibrium of physical, chemical and biological phenomena. Continual changes and zonation patterns can be observed as an outcome of pollution from various sources as described in detail below.

Organic pollution

This is one of the oldest types of water pollution in the form of discharge of organic matter into the water. The major sources of organic pollution are sewage and domestic wastes, agriculture run off from animal waste, fertilizers and pesticides, food processing, mineral, textiles and paper manufacturing industries. The major proportion of organic waste contains a high proportion of suspended matter and effects can be observed as dissolved oxygen concentration levels (Fergusson, 1990; Mackenthun, 2005).

Higgins and Burns (1975) carried out analyses of the organic component of sewage and found that typical composition of organic waste comprises lipids, amino acids, cellulose, lignin, protein, alcohol soluble fraction and ash. The chemical changes due to these materials and their effects finally lead to the depletion of oxygen content of the water and substratum. Thus, the strength of effluents causing organic pollution is measured in its biological oxygen demand (BOD) expressed as mg/litre.

Nutrient pollution

Plant growth can be limited by various factors mainly availability of inorganic nutrients such as phosphate (Moss, 1988, Abel, 2002), and light and physical characteristics. The discharge of the above discussed organic pollutants when decomposed provides the required phosphate, nitrate and other plant nutrients. To a limited extent, organic pollution helps the survival of plants life; however, excessive plant growth can cause adverse effects. Abel (2002) lists four major and adverse consequences of excessive plant growth as follows:

- i. Macrophytes and filamentous algae can cause faunal alterations owing to physical changes in the habitat.
- ii. Respiration of plants growth may result into depressed dissolved oxygen levels, not only at night but also during the day if light penetration is reduced by plants density.
- iii. Several algal species and their rapid growth can affect the flora. This algal bloom creates many problems such as tainting and discolouration of the water, making water unpalatable and producing toxins harmful for fish and other aquatic organisms.
- iv. The decay of plant biomass may have same effects as input of a large quantity of allochthonous matter.

Eutrophication

The other form of pollution is the nutrient sedimentation in the lake waters, moving or static. This happens due to cyclic formation of low temperature and high temperature stratum in the lake. These strata are known as Epilimnion and Hypolimnion. Eutrophication is the process wherein the nutrient levels of lakes increase from low nutrient status (oligotrophic) to high nutrient status (eutrophic). The basic cause is accumulation of plant nutrients and organic matter at the basin level in the lakes. The effects of eutrophication would be similar for plant growth and low oxygen levels and for organisms once it reaches beyond equilibrium level (Abel, 2002).

Thermal pollution

Thermal pollution of water is caused by change in temperature. Increased temperature can cause physical, chemical and behavioural change in aquatic organisms. Examples are: high temperature reduced oxygen solubility in water and at the same time

increased biological oxygen demand of organisms; effects on the toxicity of some poisons such as dissociation of ionisable pollutants ammonia and cyanides. Thermal pollution is mainly caused by discharge of heated effluents into rivers or lakes. The turbulent and rapid re-aerating waters can withstand heated effluents so no thermal pollution happens in such cases; however, in static waters it can have adverse effects. Thus, thermal pollution cannot be generalised but it depends upon the water quality and the regional conditions (Abel, 2002).

Toxic pollution

There are about four million different chemical substances in existence; out of these the main toxic pollutants are heavy metals, ammonia – cyanides and phenols; pesticides and suspended solids. These toxic pollutants are discharged from specific locations worldwide and thus pollute specific regions. These pollutants have different toxicity and chemical behaviour and are discussed in the next section (Nibourg, et al 2013, Paprocka and Jamroz, 2012).

2.4.4 Types of toxicity

Toxicity measurements are required because many chemical substances and their by products when used for industrial, agricultural and domestic purposes become contaminants to watercourses. In case of manufacturing new compounds or formulations, it is vital to carry out toxicity testing for the feasibility of its commercial applications. The effluent treatment plants also require toxicity testing of effluents to design the treatment and removal of complex substances in effluents. The toxicity data allows one to monitor changes happening over the time in the environment and

formulate the pollution control standards. Major categories of toxicity include lethal, sub-lethal, acute and chronic toxicity. Lethal toxicity is defined as toxic action resulting in the end of life for the organism whereas sub-lethal toxicity refers to the adverse effects rather than end of organism life span. Acute toxicity refers to the severe effects in the short time or a period of few days whereas chronic toxicity is defined as adverse effects over a longer duration measurable in weeks or months (Fergusson, 1990; Abel, 2002).

2.4.5 Factors influencing toxicity

The toxicity of pollutants can be influenced by their properties such as chemical structure or molecular configuration, for example the synthetic anionic detergents group of compounds. Previous researchers have confirmed the existence of empirical relationships between toxicity and chemical structure and physico-chemical state of pollutants (Abel, 1974, 2002). In other factor influencing toxicity is environmental conditions such as water hardness, temperature, dissolved oxygen quantity and salinity which can influence both the organism and polluting substance (Calamari et al, 1980; Abel, 2002). The presence of multiple pollutants in water enables more chemical reactions and higher exposure to increased toxicity (Abel, 2002). This is quite different from many pollutants acting simultaneously (Sprague, 1970). An important formula given by Brown (1969) and Sprague (1970) to measure the toxicity of poisons in combination can be calculated in toxic units as:

$$\text{Concentration of pollutants in toxic units} = (\text{Concentration of poison}) / (\text{Lethal threshold of concentration})$$

The fluctuation in the concentration of polluting substance is unavoidable. This is because concentrations of pollutants in the water or effluents are not constant and fluctuate widely and rapidly. Biotic factors also influence toxicity, for example, some organisms are more susceptible to poisons than others. In this way, biological characteristics of the organisms can influence their response to toxic pollutants. Interspecific or intraspecific variation in susceptibility to pollutants is shown by organisms, for example the effects of size and age of fish can influence their susceptibility to pollutants (Abel, 2002).

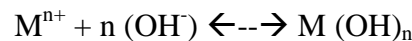
2.5 Heavy metals pollution control approaches and comparison

Heavy metals are one of the major pollution sources for water and soil. Increasing levels of these metals in the environment causes a serious concern. Heavy metals are usually defined as metals having a density greater than 4 to 5 gm/cm³. Metals such as Copper, Selenium and Zinc are essential as trace elements to human body's metabolism even if at higher concentration they are toxic (Fergusson, 1990). Other metals such as Mercury, Cadmium and Lead have direct toxic effects on human body. These metals remain in the environment changing its chemical state and getting accumulated into food chain. These metals pollution has its roots in the activities such as mining, refining and electroplating industries. This industries release effluents and increase variety of heavy metals cadmium, copper, chromium, nickel, lead and zinc. High solubility in water, mobility and recalcitrance to degradation and consequent persistence of these heavy metals make their concentration eventually increased to toxic effects state. Out of 35 metals, 23 are termed as heavy metals and chronic exposure to lead, cadmium, mercury and arsenic are major threats to human health

(Fergusson, 1990). These metals can cause numerous diseases leading to physical, muscular and neurological degenerative processes. Various methods have been developed in the recent years to remove the pollution from the waste water effluents and drinking water to improve the quality (Abel, 2002). These methods are discussed as below.

2.5.1 Chemical precipitation

This is one of the most common technologies to remove dissolve metals from water. In this method, ionic metals are converted into an insoluble form through chemical processes (Masters, 1991). The conceptual mechanism produced the form of the hydroxide. The conceptual mechanism can be represented as,



Wherein $M(OH)_n$ means insoluble metal hydroxide, M^{n+} is dissolved metal ions and OH^- precipitant. The optimum pH of one metal may affect the other metal to become soluble and go into solution. The major disadvantages of this method are the large quantity of chemicals used, and excessive effluents produced requiring further treatment. Also, slow metal precipitation, negligible settling of hydroxides, aggregation of metal precipitations and long term implications of effluent disposal are also considerations (Duggal, 2008; Zhang et al, 2009).

2.5.2 Coagulation – flocculation

Coagulation and flocculation occur in sequential processes to destabilize the suspended particles, to allow particle collision and to form the growth of flocks. Both processes must be completed to achieve pollution removal (Vesilind et al, 2010).

Coagulation occurs first to destabilize particles resulting in their sedimentation. The particle size is increased by flocculation of unstable particles into bulky floccules. The main principle in this method is preliminary adjustment of pH and addition of ferric or aluminium salts to overcome repulsion within the colloidal particles. Lime based coagulation has advantages of improved sludge settling, dewatering characteristics, capability of inactivating bacterial activities and sludge stability. The major disadvantages of this technique are high capital outlay and maintenance costs in addition to large quantity of chemicals (Duggal, 2008).

2.5.3. Flotation

This technique is applied in the liquid phase as it uses bubble attachment to separate solids. There are five different types of flotation dispersed air flotation, dissolved air flotation, vacuum air flotation, electro flotation and biological flotation. Dissolved air flotation is the one widely used to remove metals from waste waters (Vesilind et al, 2010).

2.5.4 Aeration

Aeration is required when water bodies have anoxic conditions caused by activities such as sewage discharges, agricultural run-off or over-baiting a fishing lake. Aeration is performed by infusing air into the bottom of the water levels or by surface agitation creating a fountain device to allow air and oxygen to mix with the water which would release and remove noxious gases such as CO₂, methane and hydrogen sulphide (Duggal, 2008). Aeration can be used to treat both waste water and drinking water. Aeration can be categorised into natural, surface and sub-surface aeration. For

example, in the time of heavy rain, the river Thames in London has large quantity of sewage inflows which reduced dissolved oxygen levels wherein species have difficulties to survive (Vesilind et al, 2010).

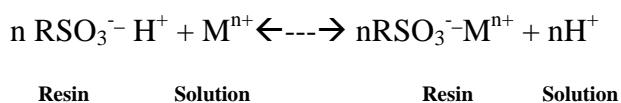
2.5.5 Membrane filtration

This technology is advantageous in terms of no addition of chemicals with a relatively use low energy and involve easy phased conduction process. This technique can help remove dissolved inorganic contaminants such as heavy metals, suspended solids and organic compounds. Depending on the particle size to be removed, many different types of filtration techniques such as ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO) can be utilized. UF utilizes 5 to 20 nm permeable membranes according to the compounds to be removed. NF mechanism is based on the steric (sieving) and electrical (Donnan) effects. It functions in such a way that the electrical effect between charged anions in membranes and metal co-ions in pollutants creates the rejection of metal co-ions through membranes and separate them. In RO, pressure application retains the heavy metals and pure water is collected on the other side of membrane. Apart from high operating costs, RO is more effective than NF and UF techniques as it removes 97% of metal between 20 to 200 mg/L (Duggal, 2008; Wang et al, 2009).

2.5.6 Ion exchange

A reversible chemical reaction of ion in the solution can be exchanged with similarly charged ion in a solid phase resin. Thus, on separation of loaded resins, metals can be removed using a concentrated solution with the help of suitable reagents. These resins

consist of sulphonic acid which facilitates the physio-chemical reaction expressed as (Wang et al, 2009),



$n\text{RSO}_3^-$, represents anionic group from resin and M^{n+} represents metal cation wherein n is the coefficient of the reaction component depending on the oxidation state of the metal ions. This method is effective between pH ranges of 2 to 6 and requires suspended solids to be removed by other methods before ion exchange process (Vesilind et al, 2010).

2.5.7 Electrochemical treatment

This technique utilizes the separation through membrane and ion-exchange as a combination. The main process is that ionized species in the solution are passed through an ion exchange membrane by applying an electric potential. These membranes are made of thin plastic materials and contain anionic or cationic charge. This technique is effective but the major disadvantages are high operational costs, high energy consumption, handling costs for sludge disposal and use of chemicals (Wang et al, 2009).

2.5.8 Use of micro-organisms

The use of nonliving microbial biomass to retain heavy metal ions is not a metabolic process and is termed as Biosorption; whereas bioaccumulation refers to an active metabolic process by living organisms. Microbial cells are excellent biosorbents because of their high surface area to volume ratios and high content of active chemo-

sorption sites. Mining and electroplating industries produce large quantities of heavy metals, using the bioaccumulation techniques these metals can be removed (Naja and Volesky, 2009 as cited in Wang et al, 2009). This will reduce the disposal problem of wastes and recovered metals can generate revenue for these industries (Mullen et al, 1992; Wang et al, 2009).

2.5.9 Microbial bio sorption

When a solid (named sorbent) interacts with chemical species (sorbate) dissolved in a liquid phase (normally water) the resultant effect is the removal of sorbate depending on the efficiency of the sorbent (Kotrba et al, 2011). If this sorbent is a biological material then such process is called bio sorption. The quality of absorbing material can be known by comparing its capability to remove sorbent from water with other sorbent removing materials reported in the literature. It is measured in the unit of the metal uptake which is defined by the amount of sorbate bound per unit of sorbent. It is expressed in milligrams of metal removed per gram of dry weight of the sorbent. The sorption process is influenced by temperature, pH, ionic strength of the metal solution (Wang et al, 2009; Kotrba et al, 2011).

2.5.10 Use of plants

All of the traditional physiochemical methods to remove heavy metals and pollutants from soil and water have high operational costs and uses of large amount of chemicals and nutrients creating the problem of effluent or sludge treatment. These methods can be advantageous for rapid removal in small areas (Fergusson, 1990). The emerging technology called 'Phytoremediation' uses plants to remove pollutants from the

environment (Salt et al, 1998; Alkorta and Garbisu, 2001) and is preferred over traditional methods because it offers site restoration, partial decontamination, and maintenance of biological activity and physical structure whilst being potentially cheap, visually unobtrusive and with a possibility of bio-recovery of metals (Baker et al., 1991; 1994). Because of these advantages phytoremediation is considered as a 'green', sustainable pollution removal process. Phytoremediation has been divided into five sub groups:

(i) Phytoextraction: plants remove metals and concentrate them in the harvestable parts of plants (Kumar et al., 1995);

(ii) Phytodegradation: plants and associated microbes degrade organic pollutants (Burken and Schnoor, 1997; 1999);

(iii) Rhizofiltration: plant roots absorb metals from waste streams (Dushenkov et al., 1995);

(iv) Phytostabilisation: plants reduce the mobility and bioavailability of pollutants in the environment either by immobilisation or by prevention of migration (Vangronsveld et al., 1995; Smith and Bradshaw, 1972) and,

(v) Phytovolatilisation: volatilisation of pollutants into the atmosphere through plants (Burken and Schnoor, 1999; Bañuelos et al., 1997). The next section explains a detailed review of this emerging green technology.

2.6 Metal absorption by plants: 'Phytoremediation' process

Advances in science and technology have created growth of industries leading to the unprecedented disturbances in ecological cycles (Duggal, 2008). The recent introduction of anthropogenic toxic chemicals and relocation of natural materials to

different environmental components (soils, ground water, and atmosphere), have resulted into the major requirements of the self-cleansing capacity of the ecosystems (Schnoor et al., 1995; Susarla et al, 2002). The existing ex-situ methods for remediation of contaminated ground waters include extraction and treatment using activated carbon adsorption, microbes or air stripping; whereas in-situ method makes use of stimulation of anaerobic and aerobic microbial activities in the aquifers. All of these prevalent methods need large capital outlays, high recurring costs and human resources to implement. Thus, researchers, businesses and governments are seeking ways to develop cheaper and faster alternatives for to treat large polluted volumes of water, soil and wetlands (Wang et al, 2009).

Phytoremediation is a technology that utilizes plants and associated rhizosphere micro-organisms to remove or to transform toxic chemicals located in water, soils, sediments and even the atmosphere. Phytoremediation is currently used for treating contaminants such as heavy metals including petroleum hydrocarbons, chlorinated solvents, pesticides, explosives and radio-nuclides, and landfill leachates. It is reported that approximately 80% polluted groundwater is within initial 20 metres of ground level. This suggests water pollution removal can be carried out using low cost phytoremediation applications (Best et al, 1997). The research in the field of plant technology applied to remediation has increased in importance (Paterson et al, 1990; Shimp et al, 1993; Simonich and Hites, 1995; Watanabe, 1997).

The different phytoremediation processes include: physical and chemical properties modification, releasing root exudates, improving aeration by releasing oxygen to root

zone, intercepting and retarding the movement of chemicals, effecting co-metabolic microbial and plant enzymes; and decreasing vertical and lateral migration of pollutants (Chang and Corapcioglu, 1998). When contaminants or in this case heavy metals are in low quantity then phytoremediation may be the most suitable pollution removal technique in terms of cost and effectiveness. This makes phytoremediation a long term and commercially viable solution (Jones, 1991).

Main methods of phytoremediation can be categorized into in situ, in vivo and in vitro types. In-situ phytoremediation is the simplest and least expensive of all three categories as plants can be in direct contact with contaminants. This in-situ phytoremediation is applied in this study as PA plants are suspended in the heavy metals and nutrient solution in hydroponic culture.

There are many mechanisms by which live plants can remediate contamination from water and soil. During phytoremediation by live plants, the quality of the process can be affected by physical and chemical properties of the contaminants (water solubility, vapour pressure, molecular weight), environmental characteristics (temperature, pH, organic matter in hydroponics content) and plant characteristics (type of roots, system, enzymes) (Susarla, 2002). There are many mechanisms by which live plants can facilitate remediation such as phytoextraction (phytoaccumulation), phytopumping, phytostabilization, phytotransformation (degradation), phytovolatilization and rhizodegradation (Shammas, 2009). Phytoextraction is the removal of a contaminant from water and Phytoaccumulation is when contaminants are not degraded rapidly but result in an accumulation in the plant. Certain plants such as PA plants in this study

show capacity to hyper-accumulate metals, for example nickel, zinc, copper, chromium and even radio nuclides. Heavy metals hyper accumulation threshold is defined as accumulation of more than 0.1% by dry weight in plant tissue and 0.01% for cadmium. Many types of plants such as trees, grasses, higher plants may be used for phytoremediation (US EPA, 1997).

2.7 Use of reed plants in pollution control

As discussed in the previous sections, current practices of pollutant removal from water and soil are costly and consume more natural resources. For example, incineration, landfill, aeration or chemical treatment plants methods of waste disposal and heavy metals removal will need land, water, electricity and production of chemicals. Thus, pollution needs to be controlled by way of alternative methods which are less costly and easy to implement on a large scale. Continuing research in this direction has proved that many plants and trees have specific characteristic in that they can grow and, at the same time absorb, toxic and heavy metals in their roots, stems or leaves. Phytoremediation is one such green technology which uses plants to remediate soil, water, sediments and surface with toxic metals, organics and radionuclide (Pradhan et al, 1998). This technique is effective, less expensive and non-intrusive as compared to other prevalent mechanical and chemical approaches (Bollag et al, 1994).

Phragmites australis (PA plants) also known as reed plants or common reed, is a plant species widely distributed and found in wide range of habitats in the world such as salt exposed wetlands, and are abundant in marshy areas and swamps (Lissner et al,

1999). PA plants are a perennial and emergent aquatic grass easily available in habitats around the world (Brix and Cizkova, 2010). Previous research has been carried out for analyzing metal trace elements in PA plants (Baldantoni et al, 2008), effects on the growth of PA plants with and without nutrients such as exposure to N, P, K (Tylova et al, 2008A), effects of heavy metals uptake such as Zn (Tylova et al, 2008B), impacts of salt stress like NaCl, Na₂SO₄, NH₄⁺ -N (Pagter et al, 2008; Tylova et al, 2008B), Zn and Cu heavy metals distribution and effects on plant growth, phenology, accumulation, biomass content and behaviour of rhizome, stem, root and leaf (Saltonstall et al, 2007; Unamuno et al, 2007; Vymazal et al, 2007; Tylova et al, 2008A) and types of reed plants such as phenotypic, genotypic, evasive reed plants in constructed and natural wetlands (Curn et al, 2007; Pengra et al, 2007; Vymazal et al, 2007).

According to the available data, PA plants vary widely based on their salt tolerance (Lissner and Schierup, 1997; Hootsmans and Wiegman, 1998), which may be due to high plasticity or other genetic factors (Brix, 1999; Clevering and Lissner, 1999). Pagter et al (2008) analyzed osmolality, chlorinity and proline in PA plants and found these plants are tolerant to NaCl and Na₂SO₄. This is facilitated by restricted uptake and transport to the leaves of Na, S and to some extent Cl, the ability to maintain a relatively high net photosynthesis, the capacity to enhance the intrinsic water use efficiency and production of proline for osmotic adjustment. NaCl and Na₂SO₄ affect the gas and water exchange processes in photosynthesis. When PA plants are treated with Na₂SO₄ and NaCl, plants do not control root contents of Na and Cl efficiently

and high external concentrations of Na can impair uptake of other cations. Also, NaCl is more phytotoxic than Na₂SO₄ (Pagter et al, 2008).

The PA plants can therefore withstand extreme environmental conditions due to their stable morphological, physiological and ecological characteristics and will grow the presence of toxic heavy metal such as Zn, Cd and Pb (van der Werff, 1991). Reed plants are known for their high detoxification and phytoremediation characteristics and have been widely used to create wetlands in the form of large reed beds for the treatment of industrial wastewater containing heavy metals (Jean and De, 1997). With the advancement of Phytoremediation technology and greener alternatives need for pollution removal, recently the attention has been focused on PA plants responses to heavy metal stresses (Fediuc and Erdei, 2002; Jiang and Wang, 2007).

Tylova et al (2008A) compared two co-occurring dominant wetland helophytes and potential competitors: *Phragmites australis* and *Glyceria maxima*. These plant species were cultivated under N and P availabilities simulating tropical wetlands with different fertility - oligotrophic and eutrophic. Long term outdoor cultivation was performed to characterize the extent to which nutrient enrichment affects plant growth, phenology and accumulation of N storage compounds in below ground organs of the wetland rhizomatous plants prior to the winter dormancy. Considering the formation of N reserves, the difference in accumulation of below ground N standing quantity by Reed plants and *Glyceria* was negligible. The annual productivity was same in both species however they differed in the distribution of biomass among belowground storage organs (rhizomes and roots). Reed plants allocated more

biomass into rhizomes which was approx. 16 to 49% of total plant dry weight as compared to 7 to 12% by *Glyceria*. In both species, relative proportion to belowground organs gradually increased during autumn. This is shown in the graph below. Values given are means ($n = 4$) at each harvest date and shown for total plant biomass ($n = 4$). Harvesting was done in April, September and December.

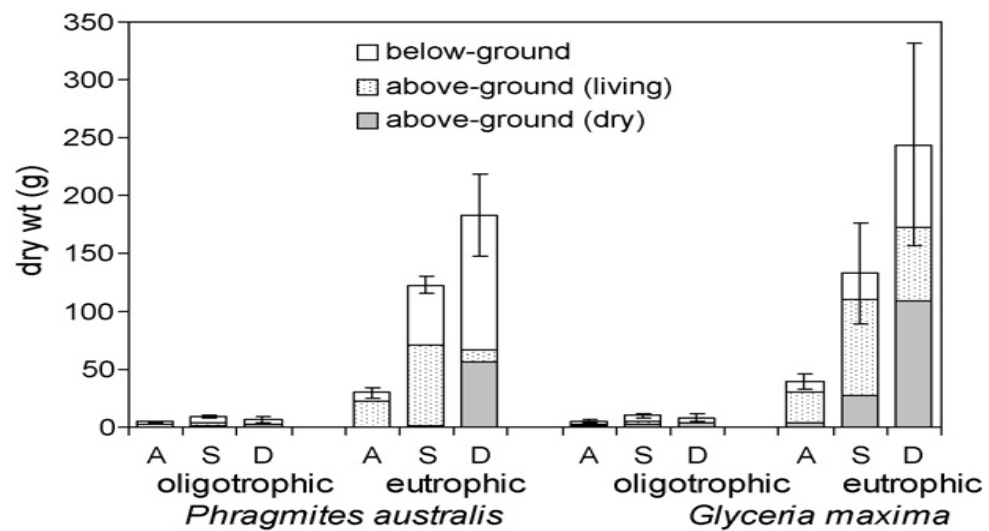


Figure 2.1 below ground organs increase in PA and *Glyceria*

(Source: Tylova et al, 2008A)

The effects of NH_4^+ and $\text{NH}_4^+\text{-N}$ show that PA has more tolerance of ammonium compounds as well as N, P, K and salt stress than *Glyceria* plant species. This is evident due to deeper roots of *Phragmites* into hypoxic sediments where NH_4^+ is dominant ((Tylova et al, 2008B, Buttery and Lambert, 1965). The sensitivity of *Phragmites* to highly eutrophic sediments may not be the main factors compared to reed plants sensitivity to litter accumulation. Under NH_4^+ predominance, reed plants may attract high water level in spring causing mechanical damage to soil (Tylova et al, 2008B). Unamuno et al (2007) studied adsorption of Cu by PA plants in the constructed wetland in which PA plants are utilized for waste water treatment and annual above ground biomass production as high as 1–2 dry kg/m^2 of land is reported. In this case, reed plants are part of solution for waste water treatment however, if not harvested at the end of the season after maximum possible uptake of toxic and heavy metals then they get decomposed and the resultant biomass litter increases the toxicity of wastewater (Gessener, 2001; Rousseau et al., 2004). Unamuno et al (2007) found that initial adsorption of Cu is high in the *Phragmites* biomass litter but after 50 minutes it reduces considerably and is possible to explain by pseudo –second order kinetic equation only and not by first order or Elovich equation. This can be due to the fundamental aspects of transition metals as characteristics of plants and metals both affect this metals adsorption process by reed plants.

During the transition passage of metals from solution to plants, only minute proportions of free hydrated ions exist and any movement within the cell involves exchange reactions between ligands, this is true for any plant which has metal uptake

capacity (Clemens et al., 2002; Outten and O'Halloran, 2001). This metal uptake capacity can be increased by roots when they release solubilizing agents which acts as a catalyst to this process in the rhizosphere since many of these agents are metal specific ligands (Campbell et al, 1988). In addition to these agents, roots release sugars, amino acids, organic acids, nucleotides, flavones and enzymes (Wang et al, 2009). Other factors influencing this process are stability of chelates of different metals varying with pH. Thus, agents released by roots in chelating many different metals are usually pH specific (Crowder, 1991). Metal uptake varies with nutrient content as well, for example, accelerated uptake of Fe is affected by the release of phytosiderophores (Fergusson, 1990; Abel, 2002). Constructed wetlands with horizontal subsurface flow (HF CWs) can be designed for treatment of sewage/effluents which can be utilized for removal of organics, suspended solids, nitrogen, phosphorus and bacteria (Vymazal et al, 1998).

Vymazal et al (2007) found in their research of waste water treatment plant in Czech republic that concentrations of heavy metals decreased in the order of roots > rhizomes \geq leaves > stems. Concentrations heavy metals such as Cd, Cr, Cu, Ni, Pb, and Zn in the above ground and below ground plant tissues were similar to those found in plants growing in natural wetlands. These concentrations were much lower as compared to plants which receive acid mine drainage, smelters effluent or highway drainage. The average leaf to stem ratio and root to leaf ratio of metal concentration was 1:5. The uptake of metal concentration can be considerably enhanced by the increased nutrient availability in the introduced PA plants. These PA plants can reach to accelerated growth during a single growing season even when established from

seeds or seedling stage. This means management of young, newly introduced PA cluster can prove to be more prudent regardless of whether eutrophication is an issue (Saltonstall and Stevenson, 2007). It has been analyzed that the uptake of heavy metals Cr, Ni, Cu and Zn in a constructed wetland and found that the distance from the inlet did not affect either shoot biomass or nutrients (N, P, K and Na) or heavy metals shoot content. Except Na, Reed plants adsorb more nutrient and heavy metals concentrations as compared to *B. maritimus*, dominating macrophytes specie. Heavy metal concentration in the incoming water and in the soil was not correlated to the plant content of either species. PA plants accumulation of heavy metals was found to be increased towards the end of the growing season (Bragato et al, 2006).

2.8 Accumulation of heavy metals in plants

Accumulation of a metal is a function of uptake capacity and intracellular binding sites. Waste water or water with multi-cellular organism has complexities in terms of the tissue and cell specific differences, intracellular transport concentration, affinities of chelating molecules, as well as the presence and selectivity of transport activities which can affect metal accumulation rates (Clemens et al., 2002). In addition to these factors, processes such as mobilization and uptake from the soil, compartmentalization and sequestration within the root, efficiency of xylem loading and transport, distribution between metal sinks in the aerial parts, sequestration and storage in leaf cell impact on the metal accumulation rate in the plants (Vymazal et al, 2007).

Many plants have shown the capability to extract and to process high concentrations of organic compounds with no significant toxic effects. Some plant species have developed the heavy metal tolerance capabilities which enable them to survive and to grow in the highly metal contaminated waters and soils (Weis and Weis, 2004; Wang et al, 2009). By accumulation of heavy metals, xenobiotics and radionuclides through phytoremediation characteristics of plants, the cost effective treatment of wastewater, ground water and soil is possible. Once accumulated in the plants, these extracted heavy metals can be transformed rapidly to reduced toxicity metabolites. Also, these toxic metals can be translocated with the help of plants to surface shoots during phytoextraction, later which can be harvested, and the tissue processed by drying, ashing or composting. A further application of these plants with extracted metals is to filter metals from water onto rootsystems that is called rhizofiltration or to stabilize hazardous metal containing sites which is called phytostabilization. With accumulation of heavy metals utilizing phytoremediation process, the volume of toxic waste produced is just a fraction of what other traditional methods of landfill and incineration typically generates (Gardea-Torresdey, 2003).

Plant species which can accumulate high levels of metal content are of considerable biogeobotanical and biogeochemical research. Bonang Nkoane et al (2005) investigated Cu and Ni accumulation in the plants *Helichrysum candolleianum* and *Blepharis diversispina* to assess their potential use as mineral indicators in biogeochemical prospecting. The analysis of plant parts: roots, stem, leaves and flowers were carried out using ultrasonic slurry sampling electro-thermal atomic absorption spectrometry (ETAAS) which allowed rapid determination of Cu and Ni in

the small amounts of samples. High levels of Ni and Cu were found in the leaves and flowers of *H. candolleianum* indicating hyperaccumulation. This current study analyzed each part of the PA plants for different heavy metal uptake and transport mechanisms within plants. As explained in the next chapters the location of heavy metals in the PA plants is determined using Transmission Electron Microscopy (TEM). The heavy metals quantity absorbed by the PA plants is measured using applications of microwave digestion and Flame Atomic Absorption Spectrometry (FAAS).

2.9 Metal extraction from plants: ‘Phytoextraction’ process

The most common methods used nowadays for the determination of heavy metals in environmental samples involve highly sensitive spectroscopic techniques, such as atomic absorption spectroscopy and inductively coupled plasma-optical emission and -mass spectrometry, the drawback of these techniques is that they first require the solid sample to be transformed into solution. Sample digestion is mainly carried out by a fusion or a wet procedure based on an acid digestion with a heated mixture of mineral acids. There are different heating systems that can be used for digestion such as, sand-bath, heating plate, pressure digestion and aluminium blocks. The introduction of microwaves, with both open and closed pressurised systems, has allowed a considerable reduction in the total time of analyses as well as in the risk of sample contamination. Whereas the use of closed digestion systems is mandatory for total determination of volatile elements to avoid losses (for example As, Hg, Cr), use of open systems allows a higher sample intake and facilitates the acid evaporation to dryness, reducing in that way following analytical problems associated with high acid

concentration (Sastre et al, 2002). Microwave-assisted sample digestion is a widely used method in sample preparation, and several standard methods have been developed on the basis of this technique. To reduce contamination from the handling steps during sample preparation, Hoffman (1988) developed a one-pot method for sample digestion. In this method a quartz insert was placed inside a digestion vessel during the sample preparation stage. The sample was weighed, digested, diluted and measured in the same insert. As there are no sample transfer steps during this type of preparation, contamination was reduced considerably. Vapour-phase digestion has been used with conductive heating, and more recently with microwave heating. Vapour-phase digestion is primarily used to reduce contamination, because the reagents are vapourized during sample digestion and only the vapour from the reagents comes into contact with the sample (Eilola and Peramaki, 2009). In the current study, the researcher applied the microwave digestion method for extracting heavy metals from the plants to estimate the metal uptake by PA plants. The detailed procedure adopted by the researcher is explained in the next chapters.

2.10 Nanoparticles formation by metal adsorption

Particles in the nanosized range have been present on earth for millions of years. Recently however, nanoparticles (NPs) have attracted a lot of attention because of our increasing ability to synthesize and manipulate such materials. Today, nanoscale materials find uses in a variety of different areas such as electronic, biomedical, pharmaceutical, cosmetic, energy, environmental, catalytic, and materials applications. Because of the potential of this technology, there has been a worldwide increase in investment in nanotechnology research and development (Guzman et al.,

2006). Engineered NPs may enter the environment by a variety of different processes and pathways. NPs can enter the environment either intentionally or unintentionally. When NPs are used for water treatment or remediation of groundwater or soil, they are added in large quantities directly into the environment. Unintentional releases of NPs may come from point sources such as production facilities, landfills, or wastewater treatment plants or from nonpoint sources such as materials containing NPs. Accidental release during production or transport is also possible.

2.10.1 Biological manufacturing methods

Microorganisms can also generate metal NPs through the generation of metabolic energy by pathways involving inorganic ions that participate in redox reactions. Oxidation of Fe^{+2} results in the formation of iron oxide NPs. Different biogenic manganese oxide NPs can be formed, both inside and outside of the cell (Matsunaga and Sakaguchi, 2000). Furthermore, metal sulfide NPs can be produced by the action of sulfate-reducing bacteria (Liming, et al, 2010). Biogenic UO_2 -NPs have been observed on the surface of bacteria in the presence of dissolved uranium (Suzuki et al., 2002). Some plants have been shown to transform copper into metallic copper NPs in and near roots with evidence of assistance by endomycorrhizal fungi when grown in contaminated soil in the natural environment (Manceau et al., 2008).

2.10.1.1 Unintentionally produced NPs

Unintentionally produced NPs are formed as a byproduct of human activities. These are mostly carbon-containing particles formed during combustion processes (Anastasio and Martin, 2001). A small fraction of these atmospheric particles also consists of metal oxides (Cass et al, 2000). Metal NPs can also be formed by other human activities, for example, welding, where mainly metal and metal oxide particles, many of them in the NP range, are produced (Lee et al, 2007). A special class of unintentionally produced NPs consists of platinum and rhodium containing particles produced from automotive catalytic converters. Although most of the Pt and Rh is attached to coarser particles, about 17% was found to be associated with the finest aerosol fraction ($<0.43 \mu\text{m}$) and may therefore include NPs (Zereini et al, 2001).

2.10.1.2 Engineered inorganic NPs

Engineered inorganic NPs cover a broad range of substances, including elemental metals, metal oxides, and metal salts (Aitken et al., 2006). Elemental silver is used in many products as bactericide (Morones et al., 2005), whereas elemental gold is used for many possible applications due to its catalytic activity (Brust and Liely, 2002). Nano-Ag is one of the most promising NPs for future applications due to its antimicrobial, antifungal, and partially antiviral properties, and is mainly used in textiles and antimicrobial polymers (Blaser et al., 2008). The use of nanoscale zero-valent iron for ground-water remediation ranks as the most widely investigated environmental nanotechnological application (Nowack, 2008).

Nanoparticulate metal oxides are among the most used NPs (Aitken et al., 2006). Bulk materials of Titanium dioxide (TiO_2), Silicon dioxide (SiO_2), and Aluminium and Iron oxides have been produced for many years. However, recently they have also been manufactured in nanosized form and have already entered the consumer market. A study from Switzerland showed that in industry the most widely used metal NPs are $\text{TiO}_2 > \text{Fe oxides} > \text{SiO}_2 > \text{ZnO} > \text{Ag}$ (Schmid and Riediker, 2008). The distribution of NP in consumer products is $\text{Ag} \gg \text{Zn} = \text{SiO}_2 = \text{Ti} > \text{Au}$ (Project on emerging nanotechnologies, 2008). One of the most widely used NP in consumer products is TiO_2 , used for applications such as photo catalysis, pigments, and cosmetic additives (Aitken et al, 2006). The useful properties of nano- TiO_2 are its self-cleaning, antifouling, and antimicrobial activity, and its strong UV absorption. In Australia alone, there are more than 300 registered sunscreen products containing nanoscaled titanium dioxide (Australian Government, 2006). Another metal oxide NP that is used in consumer products is ZnO in sunscreens (Rittner, 2002).

2.10.2 NPs characteristics

NPs are usually defined as particles having dimensions of roughly 1-100 nm, a size range where unique physical properties make novel applications possible (EPA, 2007). They can be spherical, tubular, or irregularly shaped and can exist in fused, aggregated, or agglomerated forms. The fundamental properties of a material change with size and therefore also the reactivity of a particle in a chemical reaction. Minerals in the size of approximately a few to several tenths of nanometers are in the transitional range where properties are expected to be variable and to deviate from bulk behaviour (Hochella and Madden, 2005). Hematite particles with a diameter of 7

nm, for example, adsorbed Cu ions at lower pH values than particles with 25 or 88 nm diameters, indicating the uniqueness of surface reactivity for iron oxide particles with decreasing diameter (Madden et al., 2006). An investigation of Pb adsorption onto TiO₂-NPs showed that the bulk material exhibited stronger adsorption and higher adsorption capacity than the nanoparticulate material (Giammar et al., 2007). NPs can be divided into natural and anthropogenic particles. The particles can be further separated based on their chemical composition into carbon-containing and inorganic NPs. Examples of natural inorganic NPs are biogenic magnetite or atmospheric aerosols such as sea salt. Anthropogenic NPs can be either inadvertently formed as a byproduct, mostly during combustion, or produced intentionally due to their particular characteristics. In the latter case, they are often referred to as engineered or manufactured NPs.

In this research study, NPs are produced intentionally as an added value step before the disposal of extracted heavy metals in PA plants. Silver NPs are produced as they have, at present, more industry applications as compared to other metal NPs.

2.11 Biomass utilization or disposal

Once NPs are manufactured from leached solutions prepared from the polluted biomass, the resultant plant waste still contains heavy metals. The safe disposal of biomass or its utilization in industry should ensure that heavy and toxic metals do not go back into the environment and complete removal of heavy metals take place. However, the results of this thesis show that the metal concentration, with the exception of copper, in leachate samples show that higher % of accumulated metal in

roots and stems is not released by PA plants in leaching. This poses a problem of how to dispose remaining contaminated biomass. Existing methods of landfill and incineration would send heavy and toxic metals back into the environment. Thus, other industrial applications of this biomass or methods to extract metals from biomass other than leaching must be developed which is not in the scope of this research study.

2.12 Application of reed plants for commercial use

Phytoremediation is a non-destructive and cheaper technique of water pollution control; however it has not been utilized to a great extent commercially. Many pilot projects throughout USA and Europe have proven its worth in phytoremediation on a large scale. Table 2.4 shows that most of the species used for phytoremediation are higher plants (dicotyledous) and very few monocotyledous for example grasses, reeds have been studied much in detail. In these locations plants have successfully been utilized to restore sites contaminated mainly by heavy metals Cd, Cu, Ni, Pb, and Zn. In addition to the phytoremediation usage, some plants have been utilized for their potential use as vegetative cover for water pollution prevention. Based on these pilot project results, commercial applications of phytoremediation are growing. According to Glass (2000), the United States market for metal phytoremediation in world is more than US \$ 100 million by 2005. However, the market for phytoremediation of industrial waste water is relatively low. Phytoremediation as a technique is advancing, but according to Glass (2005), it **would be** accepted through social processes and information dissemination about its potential use in removal of specific contaminants (Shammas, 2009). However, Gardea-Torresdey et al (2005) claims that

phytoremediation would contribute as a major step in the treatment of hazardous waste sites in future.

Table 2.4 Examples of American and European heavy metal sites with commercial application of Phytoremediation

Location	Plants	Contaminants	Application
Trenton, NJ[132]	<i>Brassica juncea</i>	Pb	Phytoextraction
Anderson, SC[132]	<i>Populus deltoids x P.balsemifera (hybrid polar) grasses</i>	Several heavy metals	Phytostabilization
Beaverton, OR Landfield reclamation [20]	<i>Populus spp. (cottonwood)</i>	Unspecified	Vegetative cover/water pollution prevention
Ketowice, Poland [133]	<i>Brassica jimcoa</i>	Cd,Pb	Phytoextraction
Switzerland (landfield [134])	<i>Salix viminalis</i>	Cd, Cu, Zn	Phytoextraction
United Kingdom [43]	<i>Salix spp</i>	Ni, Cu, Zn, Cd	Phytoextraction Phytostabilization
Hlemyzdi, Czech Republic [43]	<i>H. annus, C. Sativa, Z. mays, C. Hallery</i>	Zn	
Dumach, Switzerland [43]	<i>Improved Nocotiana spp. Plants (tobacco)</i>	Cu, Cd, Zu	
Lommel, Belgium [43]	<i>Grasses</i>	Zn, Cd, Pb, Cu	Phytostabilization
Balen, Belgium [43]	<i>Brassica napus</i>	Zn, Cd, Pb	Phytoextraction

(Source: Gardea-Torresdey et al, 2005, p.1807)

2.13 Conclusion

This chapter provided review of theoretical and practical views regarding water pollution, methods to contain heavy metals, phytoremediation concept and use of many plant species in phytoremediation. However, it is not known that which metals can be extracted by PA plants and PA plants are hyperaccumulator of which heavy metals. The next chapters in this thesis explain the experiments set up, laboratory tests carried out and analyses of results to answer these questions.

Chapter 3 Materials and technique

3.1 Introduction

This chapter explains the basic research theme of carrying out laboratory experiments in this research study of phytoremediation of waterways through use of *Phragmites australis* and the functional applications of all apparatus, plants and chemicals utilised in the laboratory tests.

3.2 Research theme

As explained in the previous chapters of introduction, the research objectives of this study are to review the application of *Phragmites australis* (PA) plants for their phytoaccumulation characteristics to remove toxic and heavy metals from water. Further themes of research include the manufacture of nanoparticles from the biomass from residual PA plant waste, whether the production methods will release captured toxic metal and if the metal will be incorporated into the manufactured nanoparticles. Biomass disposal methods experiments are not part of this research study.

Phytoremediation is the process of decontaminating water or soil by using plants to absorb heavy metals or other pollutants used as a means of cleaning the water or soil.

Phytoextraction is the process by which plants absorb metal contaminants through their roots and store them in their upper parts.

3.3 Key components of this research study

Following definitions and brief explanation are about important materials, processes and equipment utilized in this project.

Phragmites australis (PA)

Kingdom: Plantae

Group: Monocots

Order: Poales

Family: Poaceae

Sub-family: Arundinoidea

Tribe: Arundineae

Genus: Phragmites

Species: *P australis*

Phragmites australis, or Common Reed, is a ubiquitous plant found in both hot and cold habitats around the globe. This plant is known to cause heavy leaching of nutrients from the soil and is considered as unwanted vegetation (Dushnekov et al, 1995; Bragato et al, 2006). However, these plants have an important characteristic of being able to extract heavy metals from water and have drawn considerable attention from farmers and researchers (Saltonstall and Stevenson, 2006). Seedlings are young plants of PA and take approximately one or two weeks from the germination stage to become a plant which can be used in experiments. It may take months for seedlings to reach a fully grown plant stage.

Green house

This is constructive arrangement created to grow the PA plants from seedlings while nurturing them with water and required fertilizers. The greenhouse creates the required temperature and artificial lighting provision for plants to grow.

Hydroponic system

Hydroponics is the process of growing plants in a soilless environment where the roots are kept in a nutrient solution with or without a net liquid flow. Many plants can be kept simultaneously in the equipment and be easily monitored for growth. This is explained in detail in the next section.

Phytoremediation

This process is an application of plants' characteristics to absorb the heavy metals from water or soil. This is the key process for pollution removal and the first step in the usage of plants. The major advantage of this method over traditional methods is low cost, low energy usage and less potential of pollution generation even if implemented on a large scale.

Phytoextraction

This is the another process by which researchers remove or extract the heavy metals from the plants which has been absorbed from water or soil and accumulated in the plants' roots, stem and leaves. Phytoaccumulation or Phytoextraction are members of a group of techniques utilized based on the Phytoremediation characteristics of plants. Plants utilized in the research experiments detailed in this thesis are common reed or PA plants. Phytoremediation is essentially a new technology which makes use of plants such as PA plants to degrade, assimilate, metabolize, or detoxify metals, hydrocarbons, pesticides, and chlorinated solvents (Susarla et al, 2002). The

phytoremediation mechanisms as shown in the table 3.1 below are currently in the practice.

Table 3.1 Currently practised mechanisms of Phytoremediation

Type	Chemical Treated
Phytoaccumulation/ phytoextraction	Cadmium, chromium, lead, nickel, zinc and other heavy metals, selenium, radionuclides; BTEX (benzene, ethyl benzene, toluene and xylenes), pentachlorophenol, short-chained aliphatic compounds, and other organic compounds
Phytodegradation/ phytotransformation	Munitions (DNT, HMX, nitrobenze, nitrotoluene, picric acid, RDX, TNT), atrazine; chlorinated solvents (chloroform, carbon tetrachloride, hexachlorethane, tetrachloroethene, trichloroethene, dichloroethene, vinyl chloride, trichloroethanol, dichloroethanol, trichloroacetic acid, dichloroacetic acid, monochloroacetic acid, tetrachloromethane, trichloromethane), DDT, dichloroethene, methyl bromide, tetrabromethene, tetrabromoethene, tetrachloroethane, other chlorine and phosphorus based pesticides; polychlorinated biphenols, other phenols, and nitriles.
Phytostabilization	Proven for heavy metals in mine tailings ponds and expected for phenols and chlorinated solvents (tetrachloromethane and trichloromethane)
Phytostimulation	Polycyclicaromatic hydrocarbons; BTEX (benzene, ethylbenzene, toluene, and xylenes); other petroleum hydrocarbons; atrazine; alachlor; polychlorinated biphenyl (PCB); tetrachlorethane, trichloroethane and other organic compounds
Phytovolatilization	Chlorinated solvent (tetrachloroethane, trichloromethane and tetrachloromethane); mercury and selenium
Rhizofiltration	Heavy metals, organic chemicals; and radionuclides

(Source: Susarla et al, 2002, p. 650)

Accumulation of heavy metals

This term refers to the ability of the plant to uptake and store heavy metals from its environment in quantities.

Site of accumulation

Once the PA plants have absorbed the heavy metals from solution in hydroponic culture, the main objective is to find the location of the accumulation in the roots, stems and leaves of the PA plants. This is done by taking cross-sectional samples from PA plants roots, stems and leaves; and examining them in the Transmission Electron microscope (TEM).The detailed operations are explained in the next chapter.

Quantity of heavy metals

The second objective of the experiments is to find out how much heavy metals are absorbed by the PA plants from the solution during their active life span. This is done through the microwave assisted digestion and atomic absorption spectroscopy. Their detailed operations are explained in the next chapter.

Nanoparticle production

As a “value added” step between site remediation and biomass disposal, a low energy method of useful nanoparticle production has been investigated. Experiments have been carried out to assess the capability of plant leachates to act as the catalyst for nanoparticle production and also to assess the level of metal extraction during leachate preparation and their effects on the size and composition of the nanoparticles.

3.4 Apparatus and their application

The following apparatus were used in the laboratory research experiments of this study. Each apparatus is explained in terms of principle - how the machine works and its application that how it is used for this research project.

3.4.1 Propagator

The propagator is used for heating the soil on the greenhouse benches in propagating trays, in cold frames and while over-wintering the plants. In this experiment, propagator is used for growing the seedlings from the seeds of the PA plants. The propagator can provide temperature up to 40°C which helps creating conditions for the germination and faster growth of PA seedlings. The major advantage of using a propagator in this experiment is rapid germination of seedlings and successful propagation of cuttings. This allows PA seedlings to be healthier and stronger plants. It consists of a heating mat with a temperature probe. Following figure 3.1 shows the propagator set up (Bio Green, 2010).

Any plant can be grown in a hydroponic system provided it is rooted in a suitable media. There are two options for propagation: either by germinating a seed and the other is to take a cutting, also known as cloning. The method of cutting or cloning is preferred because it offers rapid growth, higher success rate and quicker maturity. The germination through seeds will need sterile media and capacity to hold the sufficient water. The common problem of propagation is to increase the water level unnecessarily in the propagating media. This higher level of water can disturb growth of the plant.



Figure 3.1 Propagator

3.4.2 Greenhouse

Use of green house is popular for various reasons but in this study, it is advantageous for raising PA plants, increasing possibilities of better growth of PA plants and easier propagation. Key elements in designing design of greenhouse are site selection, light, ventilation and workspace availability. The following figure 3.2 shows the author in the greenhouse with hydroponic system containing PA plants.



4 Figure 3.2 Author with hydroponic containing 30 PA plants in the green house

3.4.3 Hydroponic

By definition, hydroponics is an art of growing plants in water without the help of soil. Water table replaces the soil in the hydroponics and becomes the medium wherein plants can be suspended with their roots in the water substrate. The plants are supported in an inert media created using perlite, coco coir, clay pebbles or rockwool and are fed a nutrient solution. To allow efficient growth of plants, water has to be in movement and not still. The major influencing condition is the content of oxygen dissolved in the water that facilitates the healthy environment for plants. The saturated solution with oxygen increases the growth of the PA plants at faster rates because roots are able to absorb more nutrients and oxygen. Atomic absorption studies of the liquid in the hydroponic baths did not suggest that there was significant absorption onto the support media. The other design characteristics include size of the hydroponic bath, water level, plastic cube and cotton cloth to complete the system.

The hydroponic system provides the advantage of growing the PA plants irrespective of weather conditions as it is situated in the green house (www.futuregrow.biz, 2010; Accessed 27th August).

The main advantage of hydroponics is that plants do not have to develop a large root system in order to feed or to sustain their position. Whereas in the soil based growth, plants divide their energy evenly between growing upwards and growing downwards (rooting). Secondly, a hydroponically grown plant has less effort in growing because all the nutrients it requires are readily available through inert media. With this soilless system of hydroponics, one can control all the basic needs such as adequate oxygen, light, CO₂, fresh air, nutrients, water and heat. It is accepted that hydroponically grown plants are healthier, more robust and have quicker and larger growth rates. Hydroponic cultivation also nullifies the other disadvantages of digging, weeding, less area for plants, high water consumption, use of nitrate, fertilisers, herbicides and pesticides. In the hydroponic system, plants obtain 25% of their nutrients supplied through its roots from an inert media and rest 75% from the air in the form of CO₂. The correct quantity of nutrient solution and water are the key elements of success in the hydroponics cultivation. The following figure 3.3 shows the working of hydroponic with plants other than PA plants (www.futuregrow.biz, 2010; Accessed 27th August).



Figure 3.3 Working of a hydroponic culture system

3.4.4 Microwave digestion

This is a commonly used technique to digest organic material in an acidic environment with an oxygen source (hydrogen peroxide) before measuring the quantity of heavy metals in the solution. This method increases the decomposition of the sample and thus, helps more quantity of heavy metals to become soluble in the solution. This in turn allows the author to quantify exact quantity of metals through other techniques. This method can be applied with variations in the type or quantity of acids used or pressure – temperature combinations. The principle of microwave digestion technique includes exposing a sample to a strong acid in a closed vessel and increasing the pressure and temperature through microwave irradiation. Increased pressure and temperature of the low pH sample thereby increases the speed of thermal decomposition of the sample and solubility of the heavy metals (Kingston and Jamie, 1988). The combination of microwave digestion and FAAS methods were applied by Stolzenburg and Andren (1980) to determine ten elements in ambient aerosols and

they found accurate results which supports the use of such methods in this study. Pantuzzo et al (2009) tested three different types of microwave digestion methods with open / closed vessels and combinations of acid whilst determining arsenic quantity in complex gold mining residues. These methods included mixtures of HCl-H₂O₂-HF and HCl-H₂O₂-HNO₃. They found high pressure – open vessel technique had statistically different results as compared to medium pressure microwave sample due to incomplete dissolution. In this study, the author investigates amount of heavy metals absorbed by PA plants using flame atomic absorption spectrometry (FAAS). Hence, microwave digestion procedure acts as a catalyst or complementary technique for extracting heavy metals by PA plants.

Microwave digestion was applied in this study using the following procedure. Microwave digestion was preferred over a conventional wet digestion / oxidation technique due to above cited advantages such as the reduced amount of time for sample decomposition and metals dissolution, less quantity of acid required and control over temperature and pressure of the sample. The microwave used in the sample was a CEM Mars – and the digestion vessels were XP1500.

Method adopted for microwave digestion in this study

1. All digestion vessels and 25ml flasks were washed in decon 90, rinsed with ultra pure water and washed in 30% HNO₃ and rinsed with ultra-pure (<18MΩ) water.

2. After washing the plant samples, they were oven dried for 2 days. Then, shoots (stem & leaf) and roots were separated and ground. Samples were weighed before pre-digestion.
3. Each 0.2 gm ground sample of roots or shoots was then pre-digested in 4ml of ultra-pure 70% HNO₃ and 2ml of 30% H₂O₂.
4. After pre-digestion, the samples of ground root and shoots were transferred in Teflon XP1500 vessels inside the microwave.
5. CEM Mars model which can contain a 12 vessel sample carousel was used for microwave digestion.
6. A control vessel was used to monitor the pressure and the temperature of the microwave. The microwave heating and power program chosen was based on the similar experiment by Adam et al (2003). The following conditions were maintained during single staged closed vessel microwave digestion.

Stage	Max. Power (W)	Temperature (°C)	Ramp (min)	Max pressure (psi)
1	1200	115	8	450

7. Then vessels were cooled before extracting the samples. Samples were then taken for atomic absorption spectrometry which is explained in the next section.

3.4.5 Atomic Absorption Spectrometry (AAS)

Atomic absorption spectrometry (AAS) is a useful method in the analytical chemistry field to quickly analyse the unknown materials and their composition. This method has been already validated by literature and therefore, the author's interest is limited

to the appropriate use of the AAS in the investigation (Cullen and Barwick, 2004). All techniques of analytical atomic spectrometry are able to detect atoms or ions of analyte elements only in the gaseous phase and hence, it requires prior transformation of the sample from its condensed state into the atomic vapour state. In the atomic absorption spectrometry (AAS), two types of atomizers are commonly applied: high temperate flames and electro thermal atomizers (Gilmudinov in Cullen, 2004).

Advantages and disadvantages of FAAS

Two most popular versions of the AAS are electro-thermal (ETAAS) and flame (FAAS) depending on the type of atomiser used. This study applies flame atomic absorption spectroscopy (FAAS) which is distinguished by use of flame atomizers as compared to the rest of the AAS group of techniques. FAAS instruments are rugged, reliable and capable of quantitative measurements to single digits parts per million (mg L^{-1}) concentrations and below for a large number of elements. The other capability advantage is of multi-analyte determinations with a wide range of liquid sample matrices, including solutions containing high dissolved solid contents and/or significant portions of non-aqueous solvents. FAAS instruments are inexpensive in capital outlay and maintenance since they do not require special power supplies, cooling or use of thermostats in the laboratories. Also, the accurate and precise analyses are possible without complex calibration procedures. Hence, to determine trace elements, FAAS have become first choice of scientists. The only disadvantage of flame AAS (FAAS) is that a small fraction of sample reaches to the flame and analyte atoms pass quickly through the probing radiation beam. This results into the lack of sensitivity which is taken care of in advanced version Electro thermal AAS (ETAAS)

as trace metal contamination is minimised by addition of reagents.(Tyson and Yourd, 2004).

Basic principle of FAAS

The FAAS is a rapid and precise method of analysis in which the sample is vaporised and atomized in a high temperature flame to excited plasma. Atoms of the analyte element absorb light of a specific wavelength from a hollow cathode lamp (HCL), passing through the plasma. The quantity of energy absorbed by these atoms is measured and it is proportional to the number of atoms in the light path. A light beam is passed through the flame into a monochromator and onto a detector that measures the amount of light absorbed by the atomized element in the flame. The amount of energy at the characteristic wavelength absorbed in the flame is proportional to the concentration of the element in the sample over a limited concentration range (Csuros and Csuros, 2002).

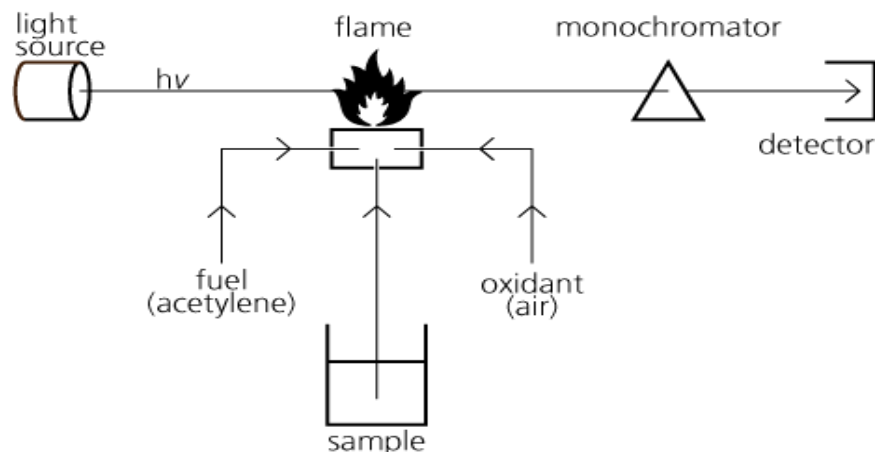


Figure 3.4 Schematic diagram of single beam FAAS

(Source: Csuros and Csuros, 2002, p.108)

The main principle of FAAS is that the extent to which radiation is absorbed by a derivative of the target analyte species is directly related to the amount of the analyte species in the material under investigation (Tyson and Yourd, 2004). Absorption spectrometry is based on a relationship between the power of the radiation incident on the absorbing medium and the power transmitted, which is formulated in terms of some fundamental characteristics of the absorbing species and their number per unit volume (or number density). The reagents in the FAAS process are air, acetylene, nitrous oxide, metal-free water and stock metal solutions. Instrumental detection limits of the heavy metals which are part of this research are given in the following table 3.2.

The underlying principle of AAS is that the logarithm of the ratio of the incident power to the intensity transmitted is directly proportional to the number of absorbers per unit volume in the beam provided certain boundary conditions are met and no absorbing species attenuate the beam.

Table 3.2 FAAS detection limits

Element	Instrument Detection Limit (mg/L)
Cd	0.02
Cr	0.02
Cu	0.01
Pb	0.05

(Source: Csuros and Csuros, 2002, p.122)

Table 3.3 Standard atomic absorption conditions

Element	Wavelength (nm)	Sensitivity (mg/L)	Linear Range (mg/L)
Cr	357.9	0.078	5.0
Cd	228.8	0.028	2.0
Cu	324.8	0.077	5.0
Pb	217.0	0.19	20.0

(Perkin Elemer AA Manual)

The current generation FAAS instrument used in the experiment has capability to do data collection and processing through software. The FAAS model used in the experiment was AS-90 model, manufactured by PERKIN ELMER. The other parameters which can influence the working of FAAS are sample introduction, nebulizers, spray chamber operation, flames, light sources, atom source emission, stray radiation, detectors, optical system, and the spectrometer and analysis effects.

FAAS has various usages in earth science, petroleum, food, beverages, clinical, forensic and the environmental science. The environmental science literature supports the use of FAAS for determination of metals such as Bi, Cd, Cr, Cu, Fe, Ni and Pb ions (Wuilloud et al, 2002; Mendiguchia et al, 2002, Manzoori and Bavili-Tabrizi, 2002 as cited in Cullen, 2004; Wang et al, 2009).

Operating process flow for FAAS during the experiments is as follows.

1. Adjust the pressures in the cylinders of acetylene, N₂O and Air.
2. Start the fan, spectrometer, computer and log on to AAS program for AA initialisation part.
3. Turn on the AA instrument; control the lamp and measure background correction, if any.
4. Not for all iterations but at the first trial, adjust the height of the burner correctly.
5. Open the flame control window, choose oxidant and adjust the flow rate with flame ignition.
6. Optimise the air/acetylene, N₂O/acetylene and burner position. This is the critical and hazardous action of the FAAS use.
7. Opt for either manual or automated analysis.
8. Check for the calibration curve. Calibration curve was obtained with standard solution for each metal – Cd, Cu, Cr and Pb.
9. Measure the standard solution to achieve the calibrated curve and then start analysing each sample.
10. Turn off the flame, air lines, acetylene cylinders, spectrometer, fan and computer.
11. Print results in the chronological orders for all iterations.

3.4.6 Transmission Electron Microscopy (TEM)

The TEM is used in this research study to ascertain the location of accumulated metals in the roots, stems and leaves of PA plants. For this purpose, cross sections of the PA plants were prepared to determine the location of the accumulated heavy metals.

The TEM operates on the same basic principle as the light microscope but it uses electrons instead of light. Resolution in the light microscope is limited by the wavelength of light whereas the TEM uses electrons as an illumination source and their shorter wavelength produces a resolution approximately a thousand times better than a light microscope. Resolution is defined as the ability to see objects as discrete individual entities. The Abbe theory of microscopy defines the limiting resolution (d) of a structure in terms of the Airy disc. ' d ' for light microscopy as shown in the following equation.

$$d = \frac{0.61\lambda}{n \sin \alpha} \sim 0.2\mu\text{m for } \lambda = 550\text{nm}$$

Where: λ = wavelength in nm

n = Refractive index

$\sin \alpha$ = Numerical aperture

Electron beams have wavelengths ' λ ' $\sim 0.004\text{nm}$ (100kV), which permit ' d ' $\sim 0.24\text{nm}$. For electron microscopy, the term $n \sin \alpha$ is fixed because the semiangular aperture $\sin \alpha$, is not adjustable and is very small and refractive index (n) is not relevant. The wavelength of electrons depends upon their kinetic energy and the above statement is a simplified derivative which accommodates aberrations such as astigmatism and spherical aberration. Chromatic aberration is very small and not correctable in most electron microscopes.

The operation of TEM

Electrons are generated from a heated tungsten source and accelerated into an evacuated column by applying a high voltage charge. The electrons are focussed onto the sample using electromagnetic lenses (condenser lenses) which also contribute to the overall resolution of the image. The sample, as an ultrathin section, is located approximately half way down the column and in the microscopes used for part of this research (JEOL 2000FX and JEOL 2100), is placed in the centre of the objective lens. The image is formed by the interaction of the electron beam and the sample. Areas of relatively high atomic number either stop the electrons or scatter them through a wide angle, an aperture placed below the sample will stop these widely scattered electrons from reaching the viewing screen or camera placed at the bottom of the column. Magnification is achieved through a combination of projector and intermediate lenses.

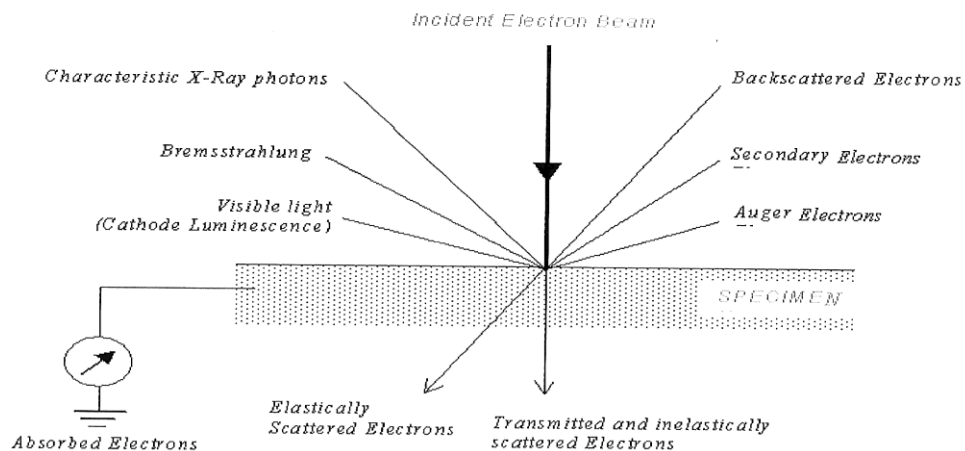


Fig 3.5 Specimen beam interactions

(Source: Courtesy of Zeiss SMT)

3.4.7 UV/Vis spectroscopy

This is used to monitor the quantitative formation of nanoparticles of metals in the PA plants leachat solution. The main principle is that UV/Vis spectroscopy detects electronic transitions occurring in molecules or ions caused by absorption of light in solution. The colour of this light depends on the wavelength of light absorbed by molecules in the solution. The colour of a solution is the result of the molecules in the solution absorbing specific wavelengths of light and transmitting the unabsorbed portion. For example, if a molecule absorbs longer wavelengths of a white light source, the solution would appear blue or green; conversely, a molecule absorbing in the shorter wavelengths would appear orange to red (Csuros and Csuros, 2002). The main components of any spectroscopy apparatus are a radiation source, wavelength selector, sample holder, detector and readout device. The schematic diagram in figure 3.6 explains the operational flow of using spectrophotometer. As shown in the figure 3.6, detector is a phototube which detects transmitted radiation through the solution and converts the light energy into electrical energy. This electric current is amplified and then transmitted to a display device. Radiation to detector is an optical component whereas detector to display is an electrical component of UV/Vis. Both single and double beam UV/Vis instruments were used in this study (Hewlett-Packard Model 8453).

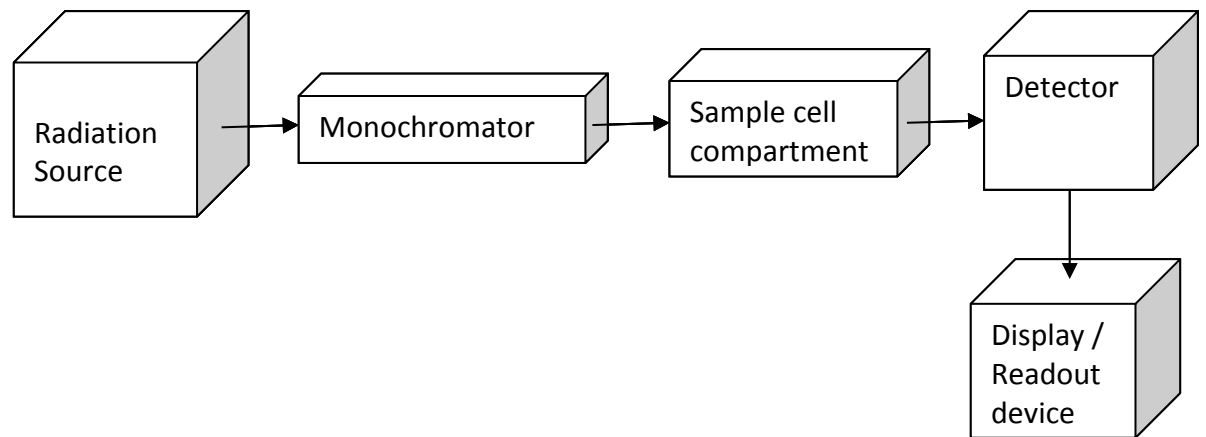


Figure 3.6 UV/Vis spectroscopy

(Source: Csuros and Csuros, 2002)

3.4.8 Zetasizer

The Zetasizer measures three characteristics of particles or molecules in a liquid medium over a range of concentrations. These three characteristics are particle size, zeta potential and molecular weight. The Zetasizer nano which was used for this study has features of pre-aligned optics, a programmable measurement position, precise temperature control, pH and concentration measurement. The results from Zetasizer were obtained in the form of correlation chart, cumulants fit, distribution, intensity; number PSD and nanoparticles size quality (Zetasizer Nano Manual 0317, Issue 5.0, August 2009). The results obtained from Zetasizer are shown in the appendix.

3.5 Plants and chemicals

Following plants and chemicals are used in the laboratory trials during this research study.

3.5.1 *Phragmites Australis*

These plants are found over the world in various habitat conditions. These are known as common reed or ditch reed in English and Qasab / Ghaab in Arabic in Saudi Arabia. PA plants are usually 3 to 4 meters tall with a stout perennial and rhizomatous reed. Their leaf blades can be 20 to 40 cm long or longer and 10 – 20 or 30 mm wide acute. Large panicle, 25 to 50 cm long and up to 20 cm in width (Milne-Redhead and Polhill, 1970). Vegetation is a major part of any wetland system and the most common plant species worldwide is *Phragmites australis* (Cav.) Trin.ex Steud (Lee and Scholz, 2007). The major characteristic found for this type of plants is their capacity to absorb large quantities of toxic and heavy metals in till their growth is affected by toxicity of metals. For example, previous researchers have studied effects of metals such as Cd, Cr, Cu, Ni, As, Fe, Pb and Zn (Jiang and Wang, 2007; Unamuno et al, 2007; January et al, 2008; Tylova et al, 2008). These plants have high potential of remediation for nutrients and metals because of their fast growth and high biomass production (Bragato et al, 2006). Generally these plants accumulate metals in the below ground tissues (Weis et al, 2004). However, phytoextraction suitability of these plants is about accumulation in above-ground or aerial tissues which is mainly affected by seasonal variations, internal growth dynamics of plants and availability of

metals and its combinations in the surrounding water and soil (Pevery et al, 1995; Weis and Weis, 2004; Bragato et al, 2006).

This phytoremediation characteristic of PA plants forms the basis of this research study and further development of their usage for waterways pollution removal. The aims of this thesis can be separated into three phases:

1. To determine the uptake of potentially toxic metals by the plant and to determine the location of these metals, if any, in the tissues of the roots and leaves.
2. To study the interactions of the metals in combination on the uptake by the plant of each metal.
3. To investigate the potential of the resultant biomass to manufacture metallic nanoparticles by extraction and whether the accumulated metals will either be leached by the preparation process and if so will they be incorporated within the manufactured nanoparticles.

Hydroponic propagation was used to grow the PA plants from its seedling stage to make them ready for laboratory trials. The following figure 3.7 shows PA plants in seedling and mature stages.

PA plants in seedling stage



Mature PA plants



Figure 3.7 PA plants in seedling and mature stages

3.5.2 Nutrient Solutions

Hoagland solution

This solution comprises nutrients for plant growth. This was used in the initial laboratory trials to see if it can help the growth and life span of PA plants so that they can absorb higher quantities of heavy metals from solutions.

Earlier experiments were carried out on *Phragmites australis* without use of any fertilizers or chemical added. Those results proved that PA seedlings could not grow beyond 15 days and could not extract much heavy metal. Hence in this set of experiments, Hoagland solution was used which acted as fertilizing agent to PA seedlings to maintain their growth while continuing to extract heavy metal. Hoagland solution consists of plant nutrients such as nitrates and sulphates of potassium, calcium, iron and manganese. Hoagland solution is prepared with following procedure and contents of different solutions as below.

- a. Make up stock solutions and store in separate bottles with appropriate label.
- b. Then add each component to 800 ml de-ionized water and filling it to 1 litre.
- c. After the solution is well mixed, it is ready to be added into heavy metal solution.

Table 3.4 Hoagland solution consists of plant nutrients

<u>Component</u>	<u>Stock solution/L</u>
2M KNO ₃	2.5
2M Ca(NO ₃) ₂ x 4H ₂ O	2.5
FeCl ₃	1.5
2M MgSO ₄ x 7H ₂ O	1.0
1M NH ₄ NO ₃	1.0

Commercial nutrient solution

For convenience, the Hoagland nutrient was substituted for a commercially available nutrient mixture for use in hydroponic culture.

Growth technology supplied the ‘Ionic Hydro Grow HW’ nutrient solution. Ionic hydro grow HW was precisely formulated as shown in its composition below. This nutrient is used for vegetative stage of growth in hydroponics and all soil-less culture (www.GrowthTechnology.com, 2010).

‘Ionic Hydro Grow HW’ is compound fluid fertiliser containing magnesium, sulphur and trace elements in the proportion of 3.5:1.2:4.6.

3.5.3 Heavy metals solution

The following metals from the heavy metals group are selected for this research study. Their solutions are prepared in different quantity and concentrations to see how much heavy metals PA plants can extract. Extracted heavy metals from plants are again separated for manufacturing nanoparticles for industrial applications.

The heavy metals solutions are prepared using the powders of the metals and their solutions of metals nitrates and sulphides. Silver, cadmium, copper, lead and zinc are selected in this study and their respective solutions used are AgNO_3 , CdSO_4 , CuNO_3 , $\text{Pb}(\text{NO}_3)_2$ and ZnSO_4 . How these solutions are made in different concentrations for experiments is mentioned in the next chapter of laboratory methods.

3.4 Summary

This chapter outlined the apparatus, plants and chemicals used in the experiments where as the following chapter will explain the methods and procedures for conducting laboratory trials using these materials. After preliminary studies, it was decided to omit zinc from further experiments as this metal is not found in excess in ground waters from Saudi Arabia.

Chapter 4 Laboratory methods and tests

4.1 Introduction

This chapter presents the laboratory methods applied to carry out the following experiments:

1. The measurement of heavy metals extracted and removed by PA plants from the water.
2. The production of metallic nanoparticles from the biomass.
3. The location of absorbed metal in the plants.

The sampling provides details about four heavy metals and their possible combinations are tested. The same procedure of explained experiment is applied to each different metal combination and concentration used in this research study. This chapter also describes the data analysis technique and software used for analysing the results of laboratory experiments. This chapter also provides justification for selection of each component of the experiments' procedure followed during the tests.

4.2 Sampling

The purpose of this government sponsored study is to facilitate a cost-effective water treatment in Saudi Arabia which has mainly these selected heavy metals (Cd, Cu, Pb, Cr) in industrial waste (Abderrahman, 2001; World Bank KSA Report, 2007) as well as to find a potential use for the contaminated biomass.

This study included heavy metals namely, Cadmium - Cd, Copper - Cu, Lead – Pb and Chromium – Cr. As discussed previously in the literature review section these metals are:

Transition metals and have multiple oxidation states. Cu and Cr are proved to be essential to the human metabolism but over–exposure to these metals is toxic for human life. Pb is a metal in group IVA (Csuros and Csuros, 2002; Wang et al, 2009).

The metal, Cd, Cu, Pb and Cr is a group of heavy metals which are most common water pollutant in the environment. The toxicity of Pb and Cd is a risk to human life even due to mild exposure to these metals and slight presence in the human body. Exceptions in this group are Cu and Cr which are essential metals in the correct quantities (Fergusson, 1990). These heavy metals have been tested for water treatment in previous research studies with numerous trees and plants as reviewed in the literature. For example, floating reed beds are used for the removal of metal pollution based on the extraction abilities of PA plants for such metals. However, it is not known whether PA plants are hyper accumulator of any of these heavy metals or not. Therefore, all possible combinations of this group of metals were utilized and their sampling combinations are shown in table 4.1. Studying combined effects of these metals on PA plants and on each other is significant in as often they collectively pollute water or soil in the natural environment. Cu and Cr are also used individually as nutrient enhancers for plants or animals but not Cd and Pb in any form for plants and animals. Also Cu is used in artificial external application, especially in Bordeaux mixture, thus Cu can permeate through soil to waterways. Cu is found in many polluted farms due to its use in animal manure as a fertilizer for enhancing growth.

Thus, future applications of PA plants for water treatment will have to remove these metals from their combined polluted solutions. Hence, it is required to analyse whether PA plants can extract and accumulate which metal and its quantity when they are combined in the solution and what effects these metals have on each other.

Table 4.1 Sampling combinations of metals

Single metals:	Each Cd, Cu, Cr and Pb
Two metals:	Cd + Cu Cd + Pb Cd + Cr Cu + Pb Cu + Cr Pb + Cr
Three metals:	Cd + Cu + Pb Cd + Cu + Cr Cd + Pb + Cr Cu + Pb + Cr
Four metals:	All metals - Cd + Cu + Pb + Cr

4.3 Seedling production

Seedlings approximately 150mm in length were purchased from Reeds from Seeds, Bryn Polyn Nurseries, Upper Denbigh Road, St Asaph, and Denbighshire, LL17 0BH. Trial experiments were conducted on these plants but as they were not available all year, a decision was made to grow from seed.

4.3.1 Seed germination

Several attempts were made to germinate *Phragmites australis* seeds in compost at room temperature but it was found that germination was limited. A heated propagator and lights were purchased from Green's Horticulture Ltd Totterdown Bridge Trading Estate, Albert Road, Bristol, UK, BS2 0XH. Seed trays were prepared using a layer of gravel covered with John Innes No.3 compost.



Figure 4.1 Propagator

Seeds were sown directly onto the compost and watered. The propagator was closed and the heating element set to 25°C and the lighting set for a 12Hr period. The propagator was set up in a dark room with the temperature controlled at 20°C. To enhance germination and early growth, the lights were fitted with white fluorescent tubes. Germination occurred within 15 days.

4.3.2 Growing on

Plants were kept in the pots in the greenhouse after the propagation stage. Greenhouse provides ideal conditions for PA plants to grow to a size convenient for use in the next stage of experiments. The following figure 4.2 shows the author in greenhouse with PA plants in the pots.



Figure 4.2 PA plants in growth stage in greenhouse

4.4 Metal concentration selection

Each single metal was tested in a range of concentrations before the hydroponic experiments to determine the appropriate concentration to be used in subsequent experiments. The metal concentrations applied to PA plants in the pilot and main experiments are shown in Table 4.2. Five reed plants were placed in each of the metal concentrations in plastic cups in the darkroom at a temperature of 20°C and a 12 hour

lighting regime. The plants were observed for 2 weeks to monitor the effects of the metal on the plant's growth.

Table 4.2 Heavy metals concentrations applied

Heavy metal	Concentrations applied in PPM for pilot tests in the laboratory				Concentration applied in PPM for main hydroponic experiments
Cd	5	10	15	25	10
Cr	1	2.5	5	7.5	5
Cu	5	10	15	20	10
Pb	5	25	50	75	50

Pilot tests were carried out on five plants sample for each single metal concentration and it was observed that plants did not survive beyond 2 weeks duration for any particular combination. To measure the extraction capabilities of PA plants further experiments with more plants in a sample and combinations of metals were carried out using hydroponic cultivation. The metal concentration selected for plants represent the highest possible concentration which can be applied to plants during growth stage. To make the heavy metals solution of different concentrations, available metal compounds such as $\text{Cu}(\text{NO}_3)_2$, $\text{Pb}(\text{NO}_3)_2$, $\text{Cd}(\text{NO}_3)_2$ and $\text{K}_2\text{Cr}_2\text{O}_7$ were utilised. Although Cr (VI) is toxic it was used due to its water soluble nature which is essentially required to study uptake of chromium by the plants.



Figure 4.3 PA plants trials with single metal concentrations

Fig 4.3 Pilot trials were carried out with different single metal concentrations in the laboratory. Five plants are kept in each concentration solution in the cup as shown in the picture.

4.5 Hydroponic cultivation

Hydroponic baths each containing 40 litres of metal and metal combinations in nutrient solution were set up in a greenhouse operated under normal seasonal diurnal lighting and at a controlled temperature of 20°C. The experiments were carried out in the spring, summer and autumn. 30 seedlings were placed in a hydroponic bath and each individual seedling was supported in machine made mineral fibre (MMMF) blocks. 40 litres of nutrient solution containing a metal, either singly or in combination (Table 4.1), was circulated around the roots of the plants using a continuous recirculated flow driven by a diaphragm pump. The solution was aerated using a porous block and an air pump and the pH adjusted to 7. All of the components

of the hydroponic system, the nutrient solution (Table 4.6.1) and pH adjuster were purchased from company named Nutriculture (www.nutriculture.co.uk).

Table 4.3 Components of the nutrient solution

Component of the nutrient solution	Component's final concentration in 40 litres of nutrient solution. Figures shown are in gm
Nitrogen	9.912
Phosphorous	3.472
Potassium	10.752
Calcium	1.96
Boron	0.0302
Cobalt	0.0016
Copper	0.007
Iron	0.12488
Magnesium	0.03528
Molybdenum	0.0036
Zinc	0.00672

Each hydroponic bath shown in this figure 4.4 contains 30 grown reed plans which are suspended in the heavy metals solution for the main experiment. Each hydroponic bath contains different concentration of heavy metal and controls.



Figure 4.4 Hydroponic cultivation in the greenhouse

Reed (PA) plants were kept in the hydroponic baths for between 4 and 6 weeks. In the initial phase of experiments, The plants were kept in hydroponic baths for 6 weeks; however, monitoring their growth during this period did not show any substantial increase in size and also showed signs of chlorosis after 4 weeks; therefore, the plants were kept in the hydroponic baths for 4 weeks only. As the experiments progressed, some plants, especially in chromium solution showed signs of chlorosis before 4 weeks and at this stage these experiments were stopped. The actual times of hydroponic culture are shown in the results section.

4.6 Preparation of plants for atomic absorption

4.6.1 Drying and grinding

After exposure to metal the plants were dried in an oven at 120° C for 2 days. Post-drying, the stem and roots were separated and ground to a fine powder by manual grinding and then using a coffee grinder.

4.6.2 Microwave digestion of plants

Ground stem and root samples of the plants were mixed with HNO_3 , H_2O_2 and water to prepare them for microwave digestion. Each 0.2 gm ground sample of roots and shoots was then pre-digested in 4ml of ultra-pure 70% HNO_3 and 2ml of 30% H_2O_2 .

This digestion process in the microwave enables the extraction of heavy metals from the ground stems and roots. The microwave MARS 5 apparatus has recommended capacity for the plant powder 0.1 gm to 0.5 gm. Thus, solution for the microwave digestion tube consists of ground stem / roots of the PA plants, HNO_3 , H_2O_2 and water.

After preliminary digestion for 12 hours, the material was transferred to microwave tubes. The microwave digestion parameters were 115°C temperature, 450 psi pressure and 1200 W power for 8 minutes. After digestion was complete, the samples were cooled to room temperature within the tubes.

4.7 Flame atomic absorption

All of the solutions available as a result of microwave digestion were analysed in the FAAS for metal concentration. The solutions which gave readings above the linear range of FAAS were then further diluted to bring them into the linear detection range available for FAAS instrument. The researcher has applied following dilution factors for each heavy metal as described below. The reading available for FAAS is then multiplied for the dilution factor to obtain the actual measurement of extracted heavy metal by the reed plants.

Table 4.4 Dilution factors and FAAS linear range

Metal	Metal concentration used in ppm	Dilution factor applied	Metal solution concentration to a level of ppm	FAAS linear range available in ppm for this metal
Cu	10	1:4	2	5
Cd	10	1:9	1	2
Cr	2.5	No dilution needed	2.5	5
Pb	50	1:4	10	20

The concentration of absorbed heavy metals in the plants can be calculated using the following equation.

$$C = \frac{c \times V \times d}{W}$$

Where,

C = Concentration of absorbed heavy metals in ppm

c = FAAS reading of heavy metal concentration in ppm

V = Volume of microwave digested solution in ml

d = Dilution factor applied from table 4.4

W = Dry weight of the plant in g

The next step after determining the quantity of heavy metals absorbed by plants is to find the location of accumulated heavy metals in the reed plants.

4.8 Location of sites of metal deposition

4.8.1 Introduction

The accumulation of metals is sub-cellular thus it is not possible or very difficult to detect their location with a conventional light microscope. Therefore, it was decided to use transmission electron microscopy (TEM) coupled with energy dispersive X-ray analysis (EDX) to determine the location of metals, if any, within the root and stem

cells of the exposed plants. The roots and stems were dissected and prepared for analysis by conventional fixation, dehydration and embedding as detailed below.

4.8.2 Preparation of Phosphate Buffer

1 litre of 0.2M **phosphate buffer** solution can be prepared as follows:

Solution A: 31.2g of $\text{NaH}_2\text{PO}_4 \cdot 12\text{H}_2\text{O}$ in 1000 ml of distilled water

Solution B: 71.6g of $\text{NaH}_2\text{PO}_4 \cdot 12\text{H}_2\text{O}$ in 1000 ml of distilled water

255 ml of Solution A was mixed with 245 ml of Solution B in a volumetric flask and made up to 1 litre with distilled water

Then mixing solutions A and B, one can obtain 0.1M Solution C as follows to prepare fixatives and resins.

Solution C: 36 ml Solution A, 14 ml Solution B and 50 ml distilled water

4.8.3 Preparation of Fixatives

Two fixatives were prepared as follows:

Fixer 1: 50 ml Solution C, 10 ml 25% glutaraldehyde and 40 ml distilled water giving a 2.5% fixative solution.

Fixer 2: 25 ml solution C, 1 vial of 0.25g of 1% of Osmium Tetraoxide (OsO_4) giving a 1% solution.

4.8.4 Preparation of resin

The recipe for preparing resin is as follows:

Resin: 24g Agar 100, 13g DDSA, 13g MNA and 1 ml of BDMA

Once the reagents are combined, the resin is placed in a desiccator for 30 minutes to remove any gas bubbles.

4.8.5 Protocol of sample preparation

1. Samples of root and stem were dissected out and cut into pieces approximately 1 mm long under glutaraldehyde fixative in phosphate buffer.
2. The samples were fixed in glutaraldehyde overnight.
3. The glutaraldehyde was removed and the samples washed in two changes of phosphate buffer for 15 minutes each.
4. Fix in Osmium tetroxide for 1 hour.
5. Wash again with buffer twice for 15 minutes.
6. Dehydrate in water or ethanol mixtures for 30 minutes.
7. 50%, 75%, 90%, 100% and 100% overnight.
8. Substitute with propylene oxide for 1 hour.
9. Infiltrate with 50:50 propylene oxide (1.2. epoxypropane) or resin overnight.
10. Embed in 100% resin overnight with open lid to allow evaporation of propylene oxide at a temperature of 65° C.

The sample preparation, except for resin polymerisation, was performed at room temperature on a sample rotator.

4.9 Transmission Electron Microscopy

Semi thin sections, 250 nm in thickness, were cut on a RMT Powertone ultramicrotome using a diamond knife. The sections were mounted onto uncoated 200 mesh copper microscope grids except when copper was present in the experiment, these sections were mounted onto uncoated 200 mesh aluminium grids. These sections were examined unstained within a transmission electron microscope operated at an

acceleration voltage of 100kV. Representative electron micrographs were taken to show the presence of electron dense particles within the cells of the roots and stems. Energy dispersive X-ray spectra were acquired to determine the elemental composition of the particles.

4.10 Manufacture of metallic silver nanoparticles

The successful accumulation of toxic metals by the plant leads to another problem. Whilst the pollution of the water has been remedied, what is done with the contaminated biomass? Burying in landfill or incineration are two alternatives of disposal but both relocate the contamination. This thesis is not concerned with the ultimate fate of this contaminated biomass or with the possibility of extracting and reclamation of the metals. However, if a step could be taken to add value to the biomass which used minimum energy to produce a product with commercial use, this may go some way to offset the costs of safe disposal. The following step-by-step process was followed to manufacture silver nanoparticles from biomass.

(i) Leachate Preparation

1g of plant root and stem (dry weight) as washed, finely cut and ground then suspended in 20ml of sterile and distilled water in Erlenmeyer flask. The solution was boiled for 5 minutes to produce a leachate which was then filtered through filter paper to remove the solids (Song and Kim, 2009).

(ii) To assess the abilities of a leachate from the control plants root and stem 10ml of leachate was added to 190 mL of 1mM aqueous silver nitrate solution for reduction of

metal positive charged ions. This is based on the manufacturing Silver nanoparticles from plant leaf extracts by previous researchers (Wang et al, 2009).

(iii) To assess nanoparticle production uv/vis spectroscopy was used to monitor the height of the plasma peak at approximated 480 nm. The measurement was conducted in either a twin beam or single beam spectrometer, 1cm quartz cuvettes were used throughout as the more conventional polystyrene cuvettes attenuate the signal at lower wavelengths.

A baseline correction was applied on each spectrum by acquiring a spectrum from 1 mM AgNO₃. The correction is automatically performed by the computer. Spectra were acquired immediately on mixing the leachate with the AgNO₃ solution and then at intervals of 5, 60, 90, 120, 150 and 210 minutes.

(iv) At the end of the experimental period, the nanoparticles formed were pelleted using an ultra centrifuge at a speed of 15000 rpm for 20 minutes. The supernatants were decanted and the nanoparticles re-suspended in several drops of deionised water. One drop of each suspension was placed onto an ultrathin carbon film on 200 mesh copper TEM grids and allowed to settle for 5 minutes. Then, excess fluid was removed by touching the side of the drop with a piece of filter paper. The grids were dried and examined within the TEM at an accelerating voltage of 100kV. Representative electron micrographs were recorded and energy dispersion X-ray spectra acquired to assess the purity of the nanoparticles.

(v) The re-suspended nanoparticles were further diluted and the particle size distribution measured by zeta potential spectrometry.

4.11 Further application and disposal of biomass

The residue of plant waste remaining after leaching and nanoparticle manufacture is considered as contaminated biomass. This biomass made up of contaminated plant waste, can be utilized for producing energy or fuel or it has to be safely disposed so as not to pollute the environment again from the extracted heavy metals embedded in the biomass. Both applications only result in the translocation of the pollutant species but the disposal of this biomass is not part of this thesis. However, the biomass with lesser content of heavy metals can be utilized for fuel or energy production purposes as it may not generate further air pollution. The production of energy or fuel can be done utilizing this biomass through three types of processes: thermal, chemical or biochemical conversion. Thus, biomass can be converted through these conversions into useful gaseous or liquid forms. These manufacturing of energy or fuel; disposal by landfill or incineration have various existing methods which are not in the scope of this thesis.

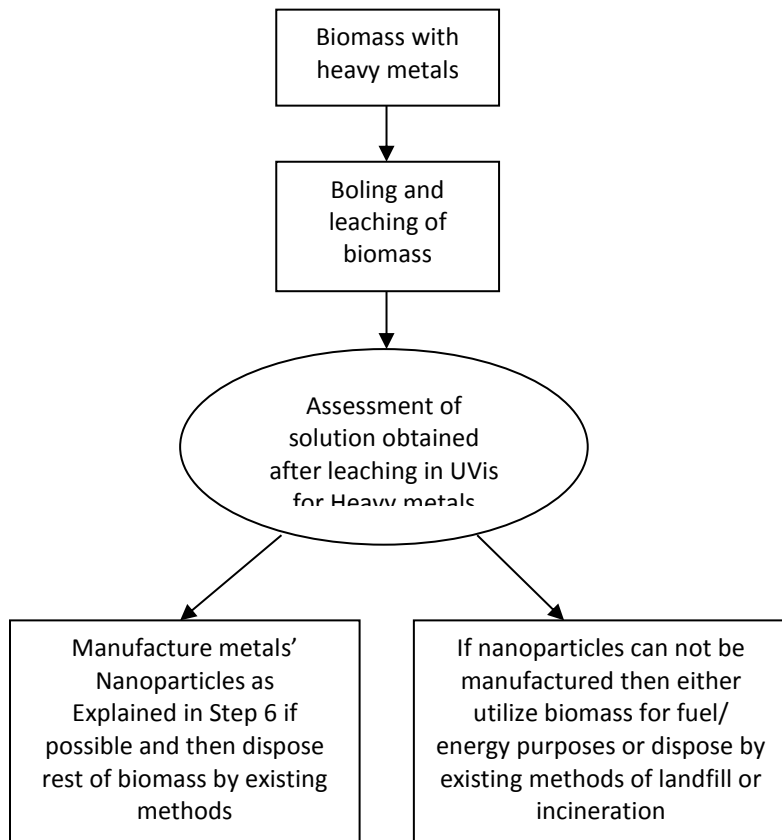


Figure 4.5 Biomass utilisation or safe disposal process

(Source: Author)

4.12 Data reporting and analysis

Data obtained through experiments are displayed in the forms of micrographs, spectra, tables and charts. The discussion of findings from the results will be related to the following points:

1. Plant growth within the metal solution – how many weeks PA plant can survive in the each metal at what concentration. This can be discussed based on the hydroponic observations.
2. The root, leaf or stem part of plant where each metal gets accumulated. This will be based on the TEM application results.
3. The quantity of each metal which can be absorbed by PA plants in hydroponics. This will be based on the results obtained from FAAS observations.

Therefore, there are various experiments results and indicators which are correlated to one another.

4. To manufacture metal nanoparticles from biomass.

4.13 Summary

This chapter discussed each stage of the experiment process and how it is carried out in the laboratory. Main stages of the experiment are hydroponic growth, TEM and FAAS observations and nanoparticle manufacturing which are discussed in detail. The next sections of thesis are based on the results obtained from the experiments done with many samples of PA plants.

Chapter 5 Results of the laboratory tests

5.1 Introduction

This chapter reports the results of the laboratory tests carried out deploying the material, methods and experimental procedures mentioned in the previous chapters.

The results are reported in three sections:

1. Metal uptake by PA plants leaf and roots;
2. Location of metal in the plants;
3. Silver nanoparticles manufactured from contaminated PA plants biomass.

Results are mainly described in the form of graphs and tables.

5.2 FAAS results for metal uptake

5.2.1 Introduction

Pilot experiments showed that the plants exposed to the metal concentrations used for the experiments for longer than 4 weeks showed signs of distress.

Therefore the PA plants were kept in hydroponic culture and samples were taken after 2 and 4 weeks duration in solutions with different metal combinations. Thus, main experiments for metal uptake measurements are carried out for 2 weeks and 4 weeks duration.

5.2.2 Experimental Results

The following charts show the comparison between different metal absorptions on a scale of 0 to maximum 100 ppm for 2 and 4 weeks duration. Metal absorption was measured in three modes from leaf, roots and leached samples. The absorption is compared with controls and all metals samples in each case. These results are the combined total of 5 plants per metal species or combination.

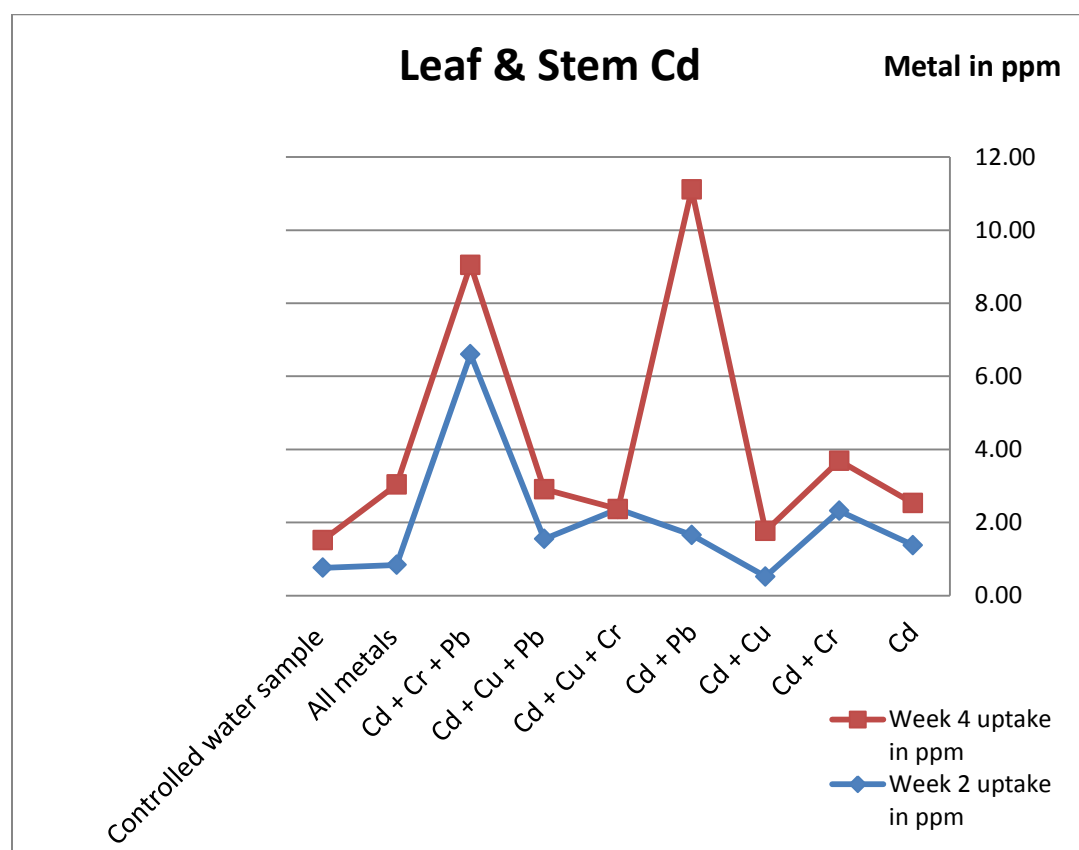


Fig 5.2.1 AA results for leafs exposed to cadmium in single or multiple metal combinations.

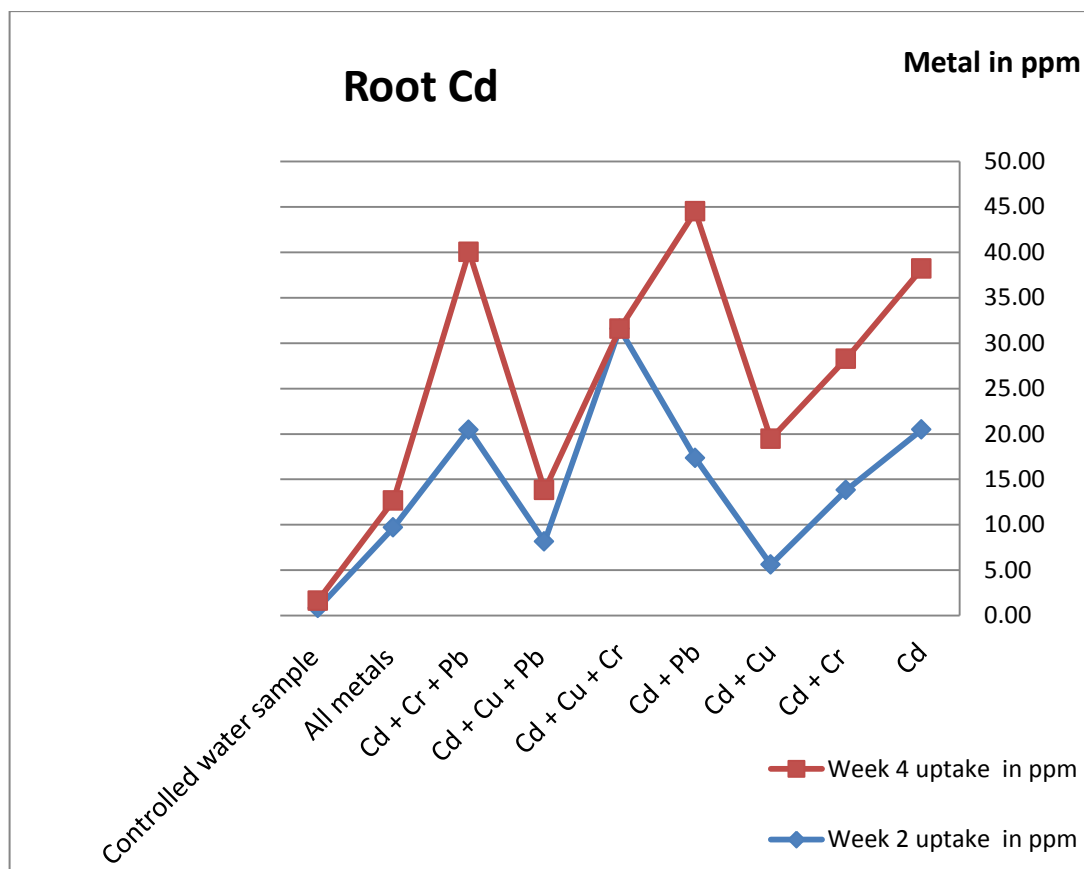


Fig 5.2.2 AA results for roots exposed to cadmium in single or multiple metal combinations.

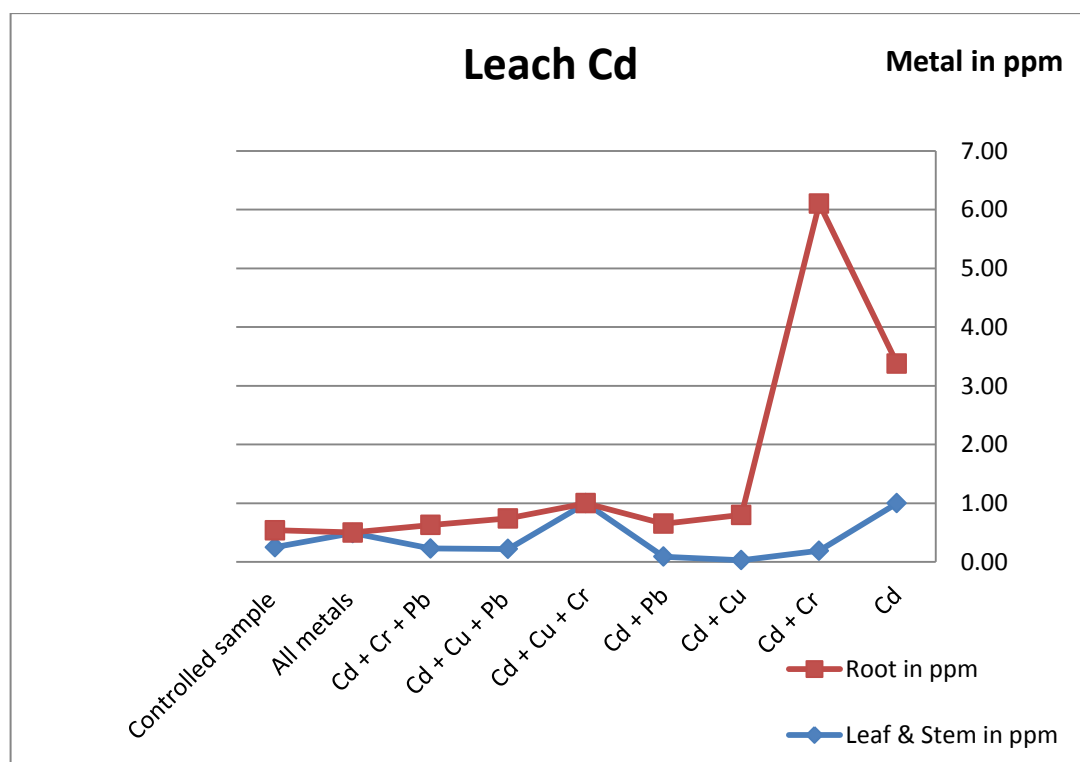


Fig 5.2.3 AA results for cadmium concentration in the leachate

The graphs Fig 5.2.1 and Fig 5.2.2 show that lead and chromium enhanced the uptake of cadmium but uptake was reduced when copper was present. The results obtained from the leaf absorption experiments is replicated for roots as the presence of lead and chromium gave the highest absorption whilst Cd+Cu, Cd+Cu+Pb and all metals have the lowest absorption. The Fig 5.2.3 for the leached samples shows that, compared with the uptake of cadmium in the roots and leaves, an insignificant amount of cadmium is released during leaching. These results are of the release of metal absorbed by the roots and leaves by leaching in boiling water.

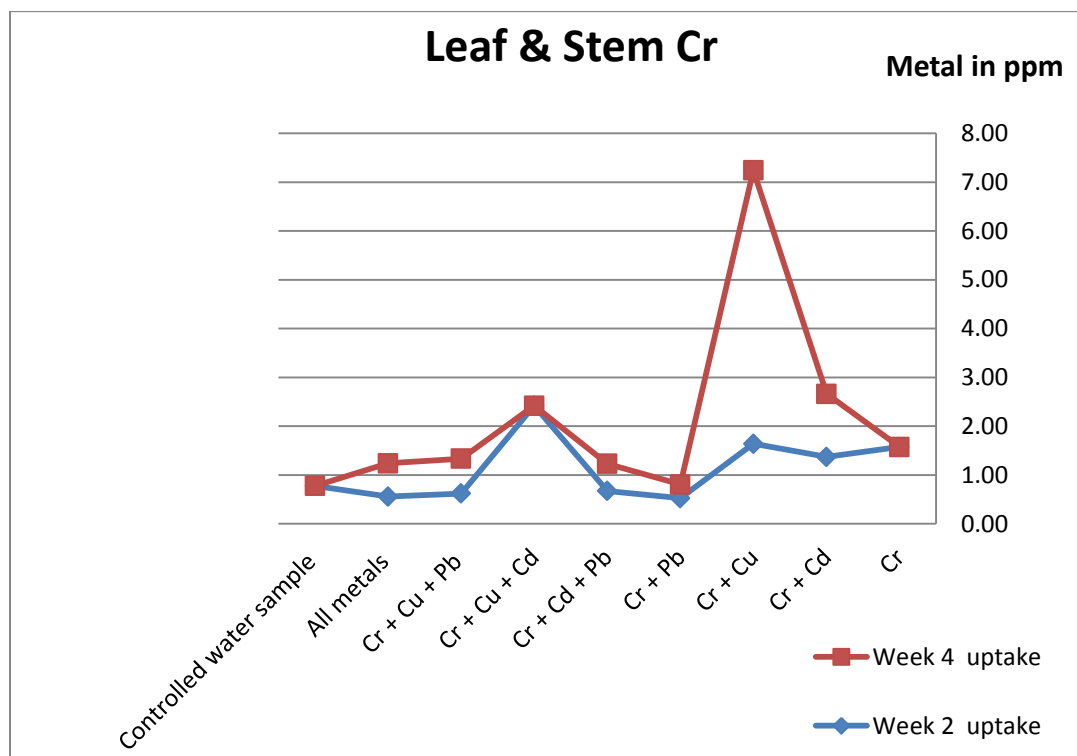


Fig 5.2.4 AA results for leaves exposed to chromium in single or multiple metal combinations

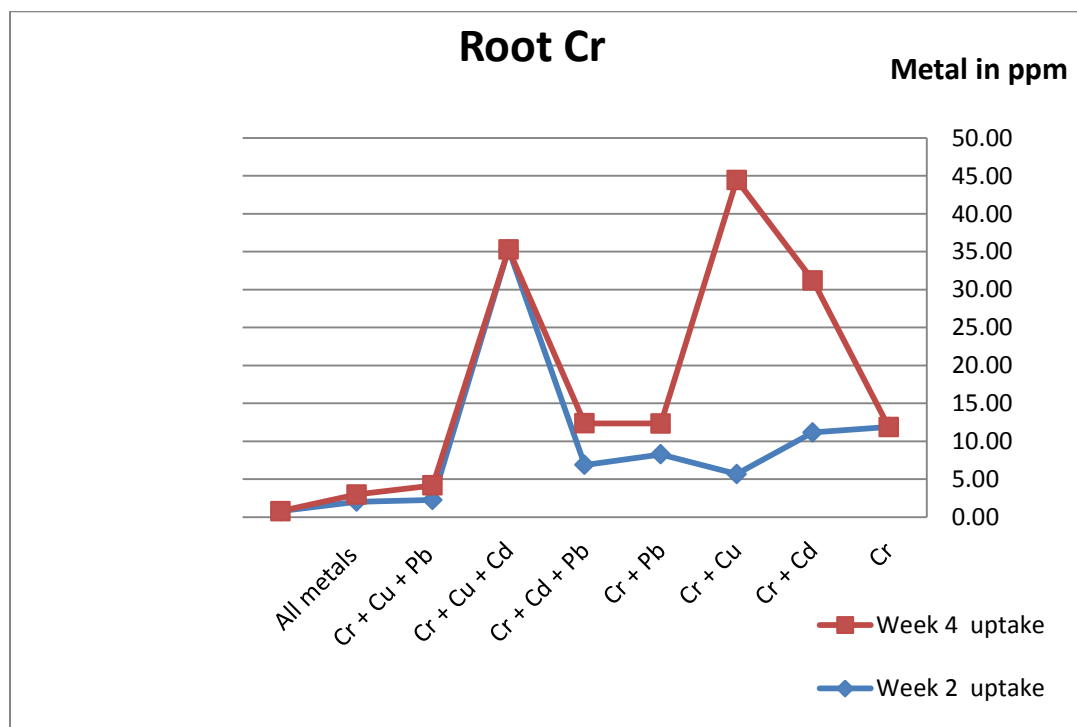


Fig 5.2.5 AA results for roots exposed to chromium in single or multiple metal combinations.

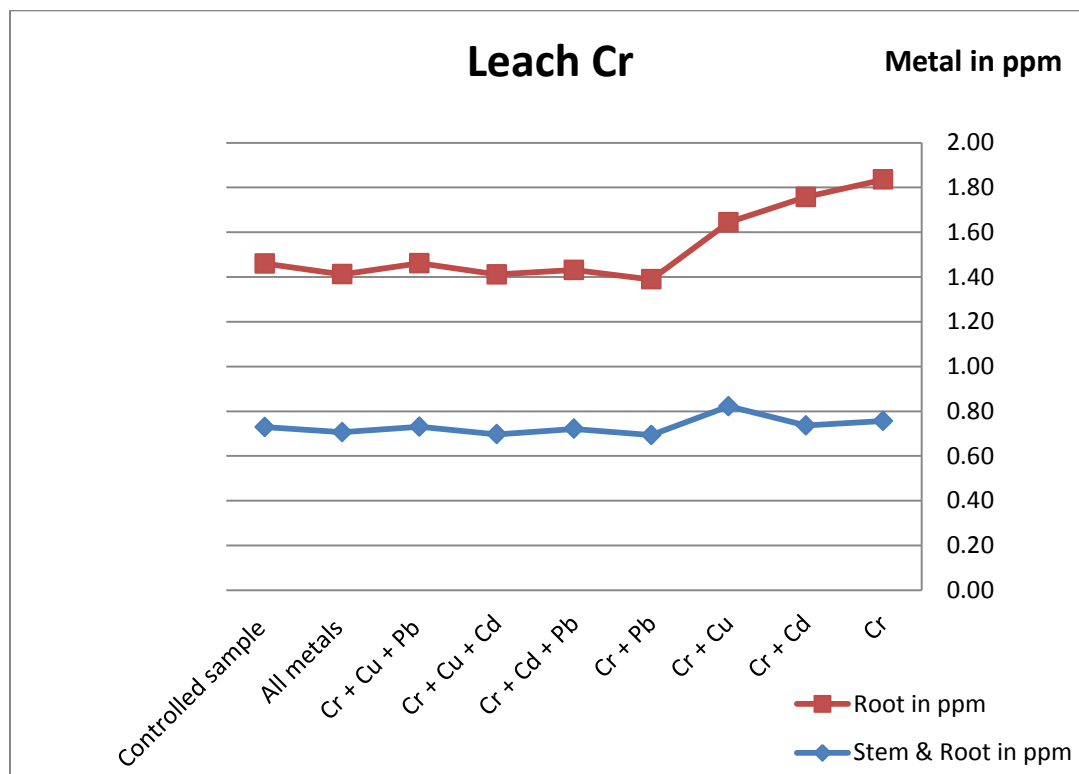


Fig 5.2.6 AA results for chromium concentration in the leachate

A fig 5.2.4 and 5.2.5 show the chromium concentrations where copper appears to enhance the uptake of chromium whilst lead appears to inhibit uptake. The Fig 5.2.6 shows that like cadmium, chromium is not leached from the samples.

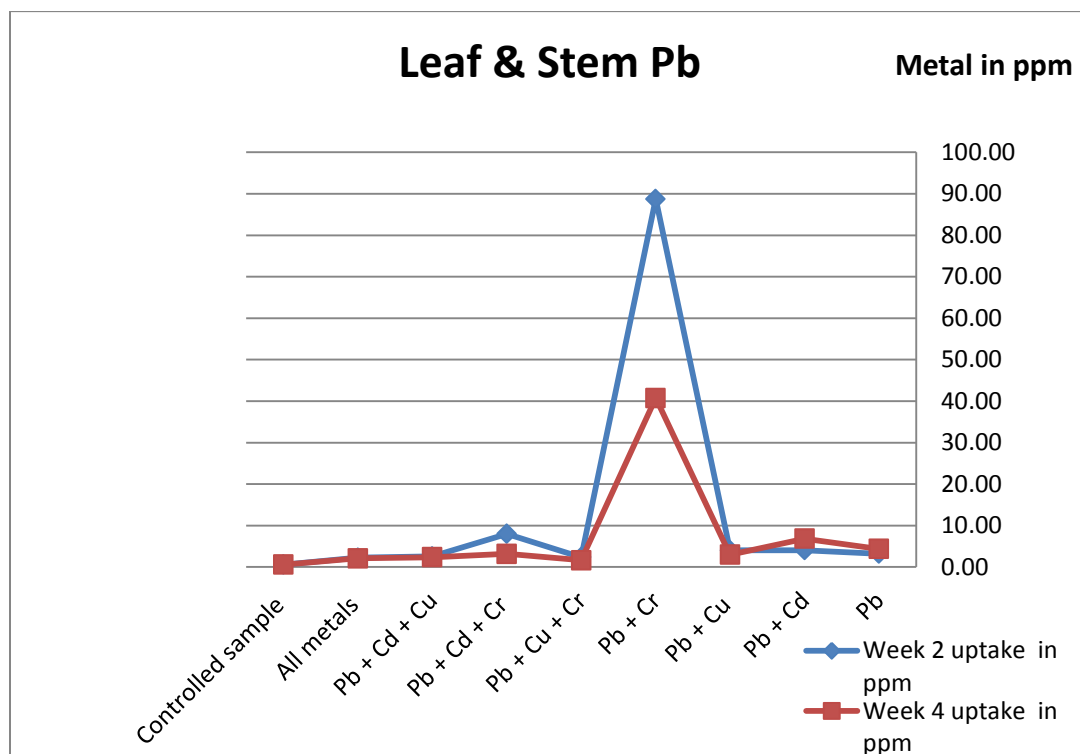


Fig 5.2.7 AA results for leaves exposed to lead in single or multiple metal combinations

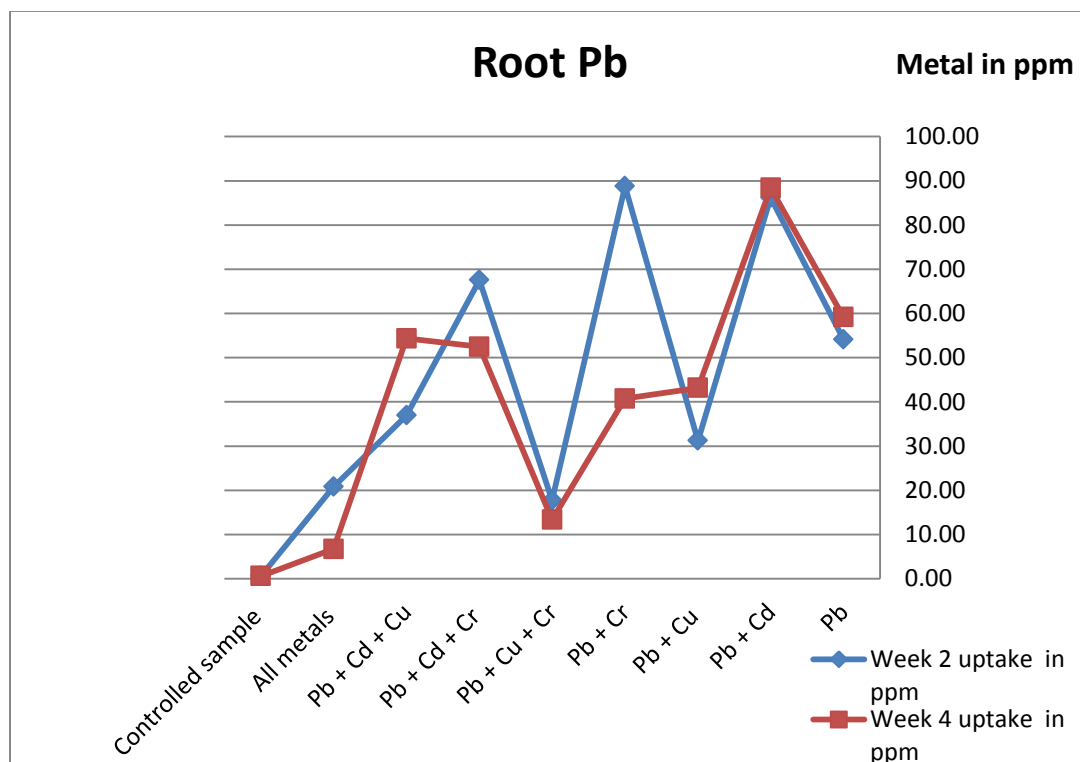


Fig 5.2.8 AA results for roots exposed to lead in single or multiple metal combinations.

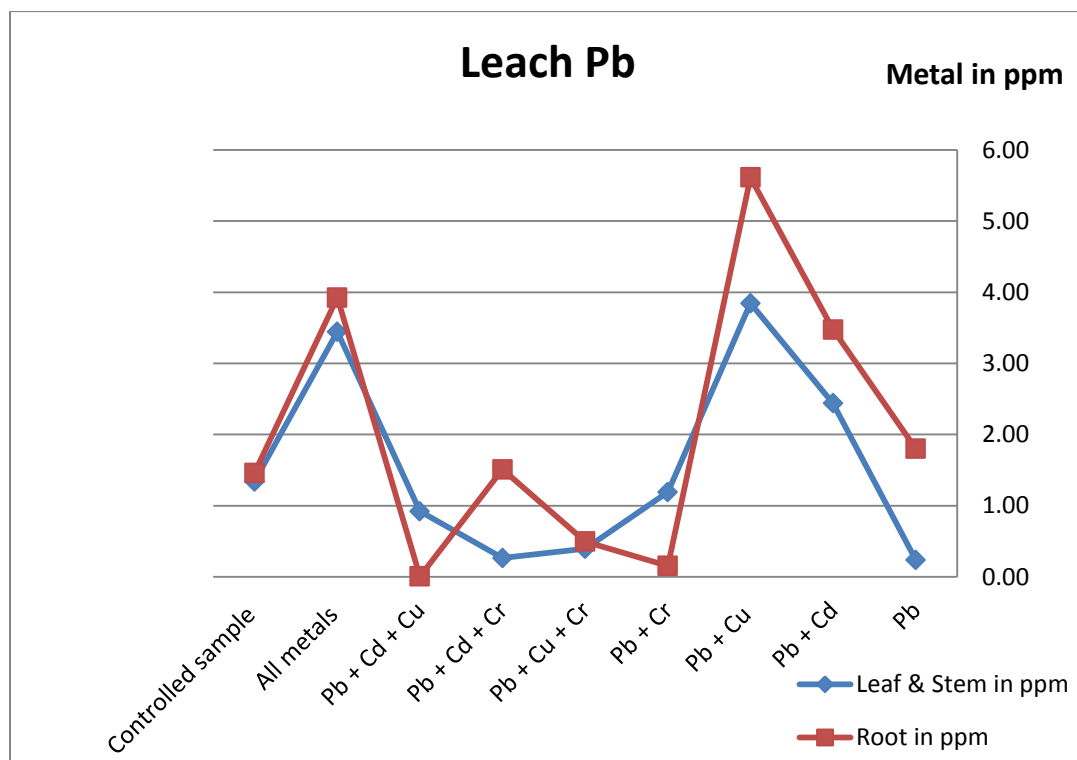


Fig 5.2.9 AA results for lead concentration in the leachate

Graphs 5.2.7 and 5.2.8 show a mixed response of lead in combination with other metals. In the leaf, chromium appears to greatly enhance the uptake of lead, where as on its own and in combination with cadmium and copper, it is not significantly taken up by the leaves. In the roots the pattern of uptake is complicated and does not show any trends. In the leachate, little lead is accumulated (Fig 5.2.9).

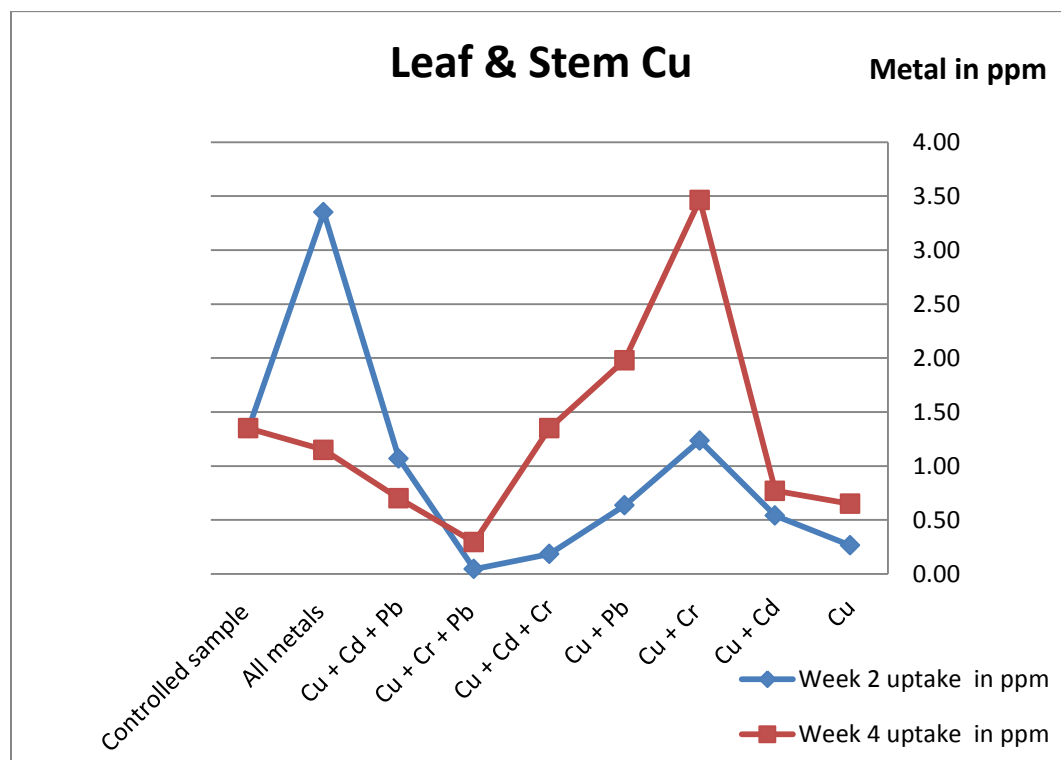


Fig 5.2.10 AA results for leafs exposed to copper in single or multiple metal combinatio

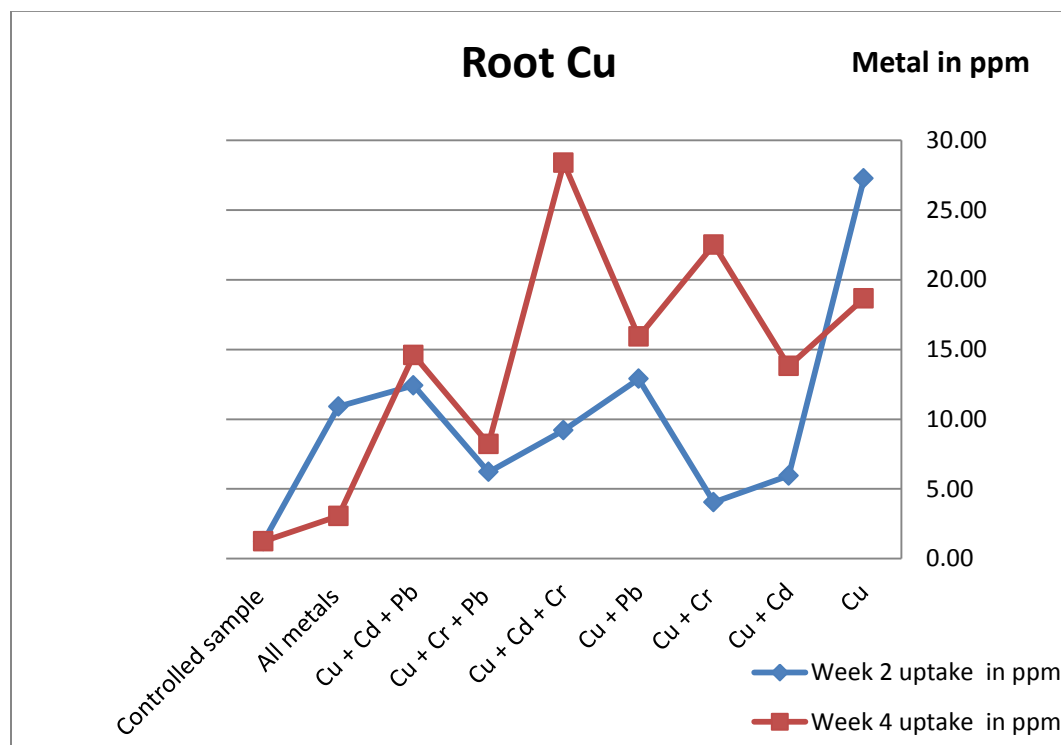


Fig 5.2.11 AA results for roots exposed to copper in single or multiple metal combinations.

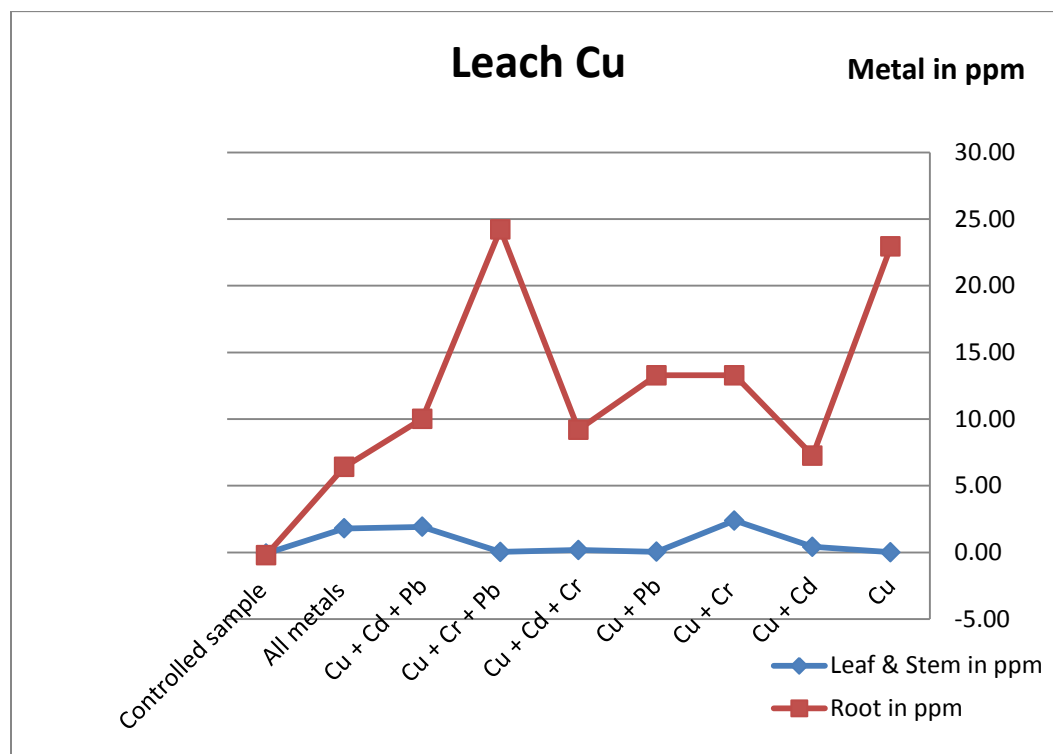


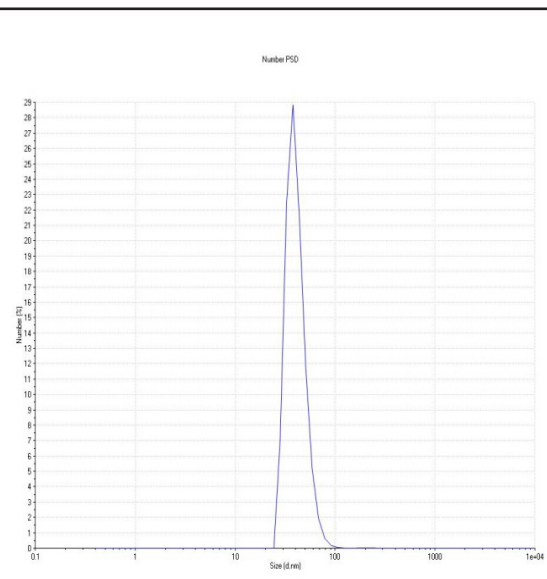
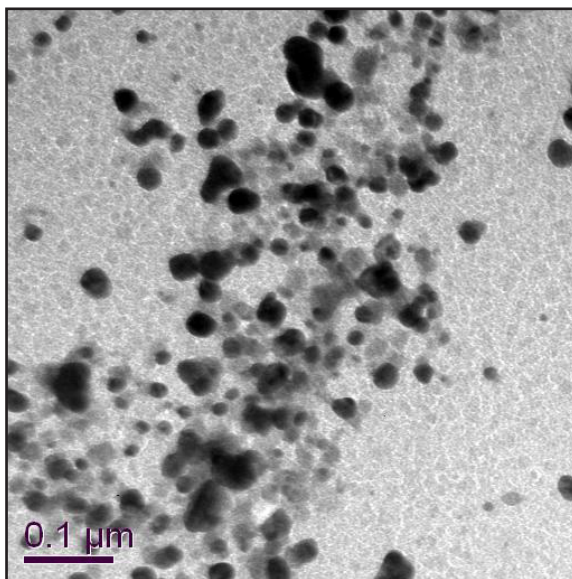
Fig 5.2.12 AA results for copper concentration in the leachate

It can be observed from the graphs 5.2.10 for uptake by the leaves, copper is not significantly absorbed. Fig 5.2.11 shows that for the roots, the absorption of copper does not show a consistent pattern of absorption when combined with other metals. The leachate (Fig 5.2.12) shows a significant release of copper from the tissues.

5.2.3 Discussion

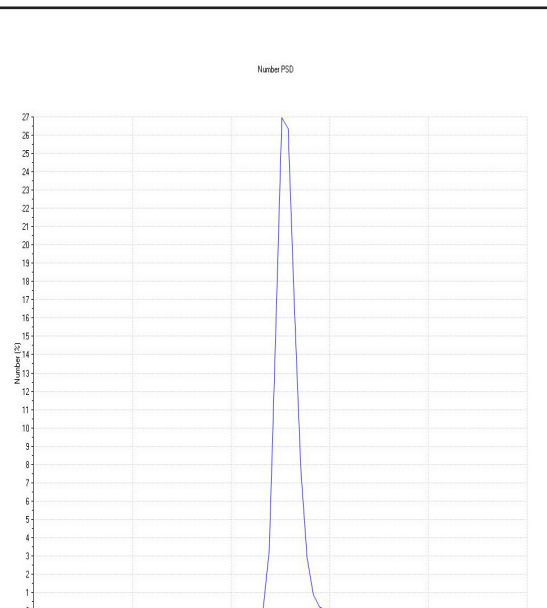
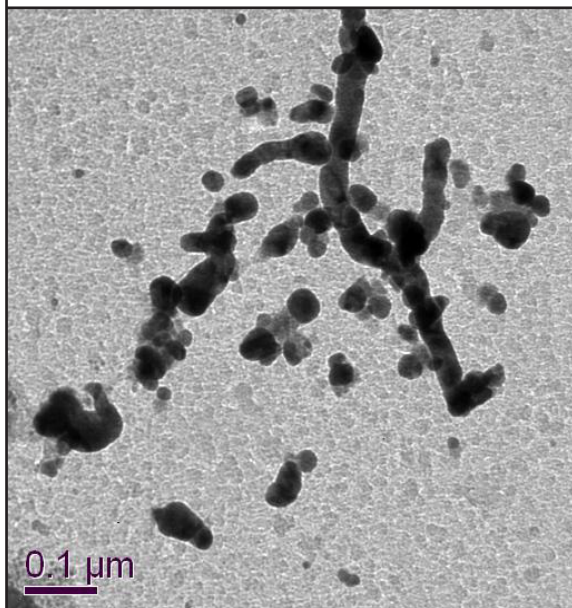
The results obtained from the flame atomic absorption experiments show a complicated relationship between metals. In all cases, apart from the combination of chromium and copper, the leaf samples did not accumulate significant amounts of metal. However in some leaf samples there was an unexpected cadmium result of 10ppm that may be due to certain error in measurement. In the roots, the results show that in general there is not an apparent pattern of either enhanced or decreased absorption from one metal to another. The experiments using a mixture of all four metals consistently showed a poor accumulation of any metal in either the roots or leaves compared with other combinations or pure metals. The leachate experiments showed that copper is released into solution and this phenomenon will be investigated in the nanoparticle production experiments. Copper appears to have an effect on the absorption of other metals; copper ions affect the cell membrane making the desorption of other metals possible.

5.3 TEM images of nanoparticles and size data



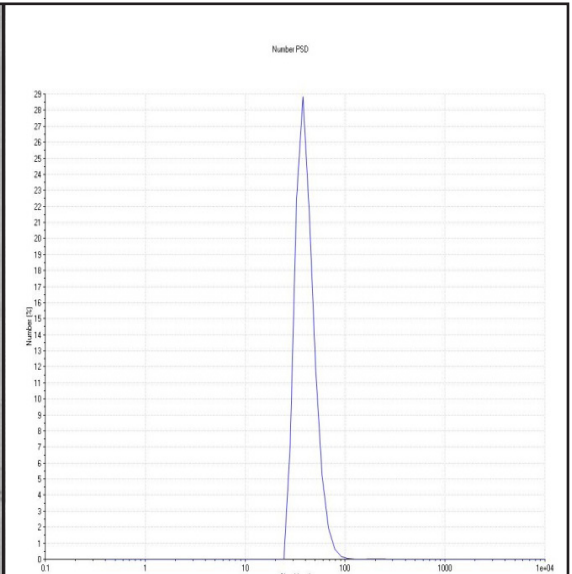
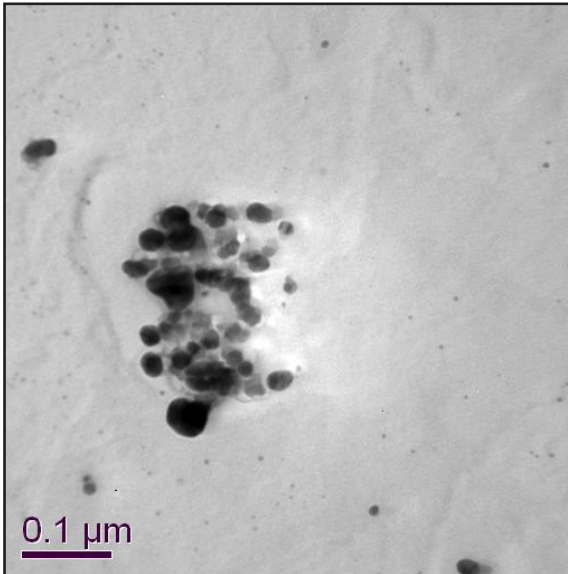
5.3.1a Silver nanoparticles from leachate from leaves grown in copper solution

5.3.1b Particle size analysis



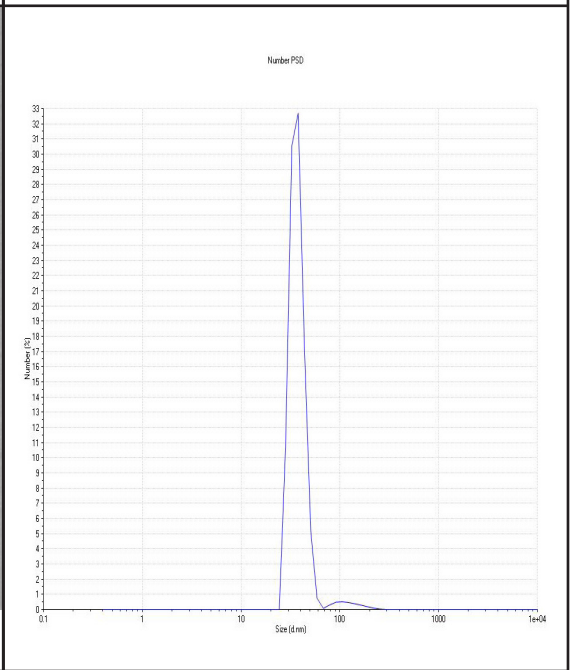
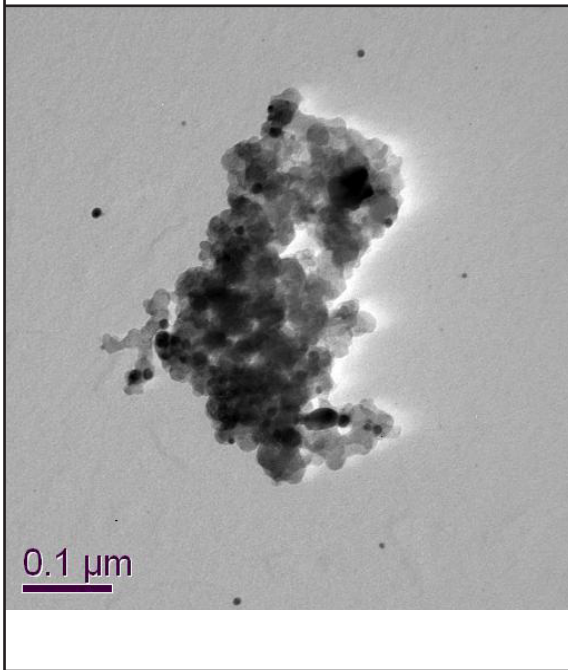
5.3.1c Silver nanoparticles from leachate from roots grown in copper solution

5.3.1d Particle size analysis



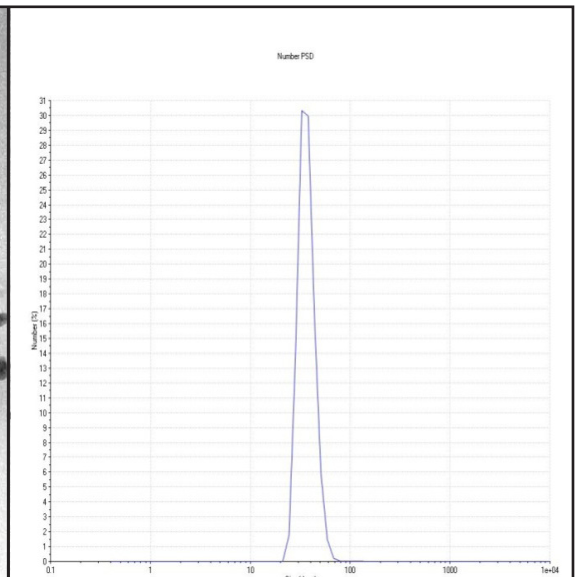
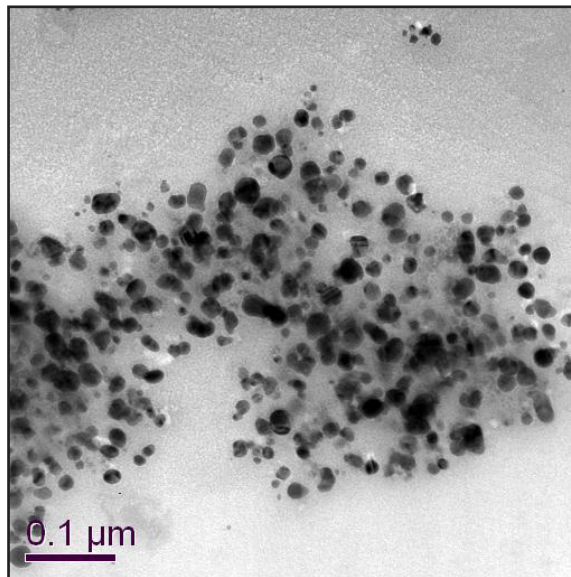
5.3.2a Silver nanoparticles from leachate from leaves grown in chromium solution

5.3.2b Particle size analysis



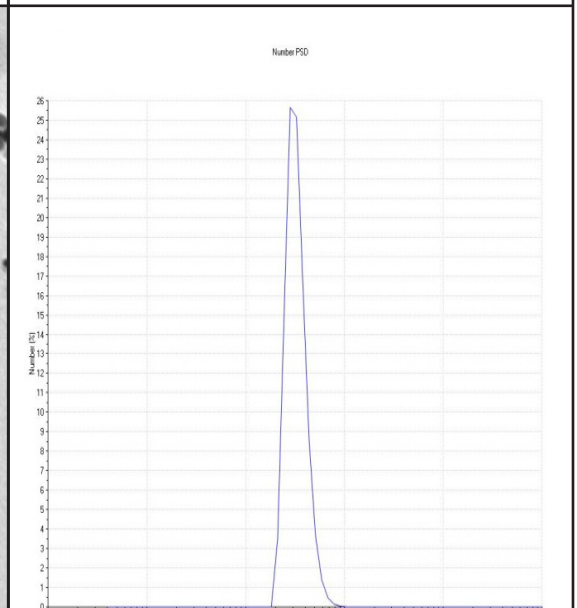
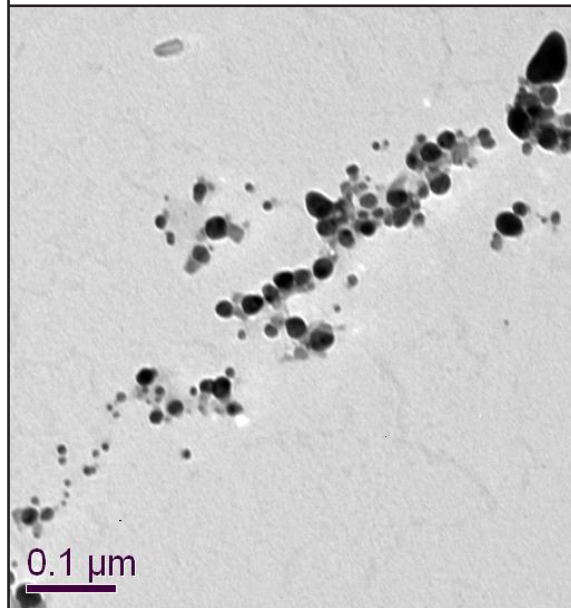
5.3.2c Silver nanoparticles from leachate from roots grown in chromium solution

5.3.2d Particle size analysis



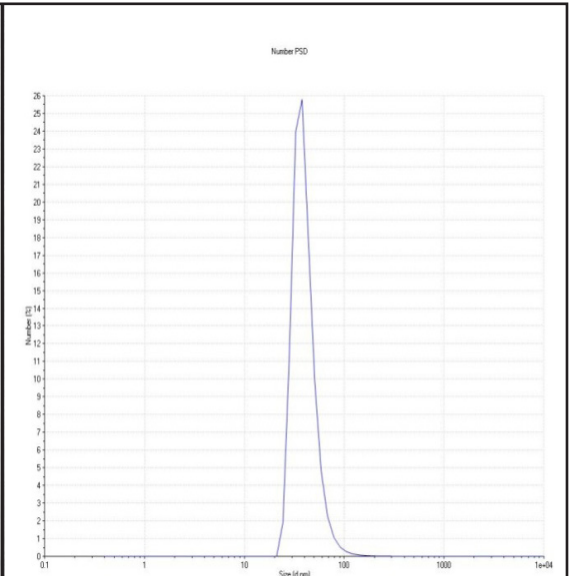
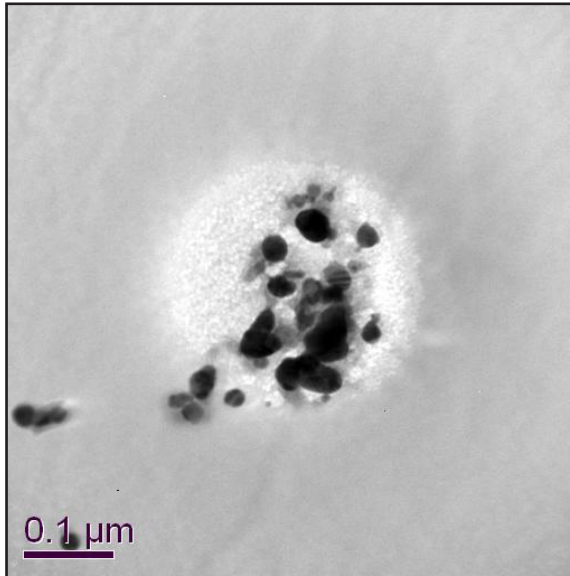
5.3.3a Silver nanoparticles from leachate from leaves grown in cadmium(Cd) solution

5.3.3b Particle size analysis



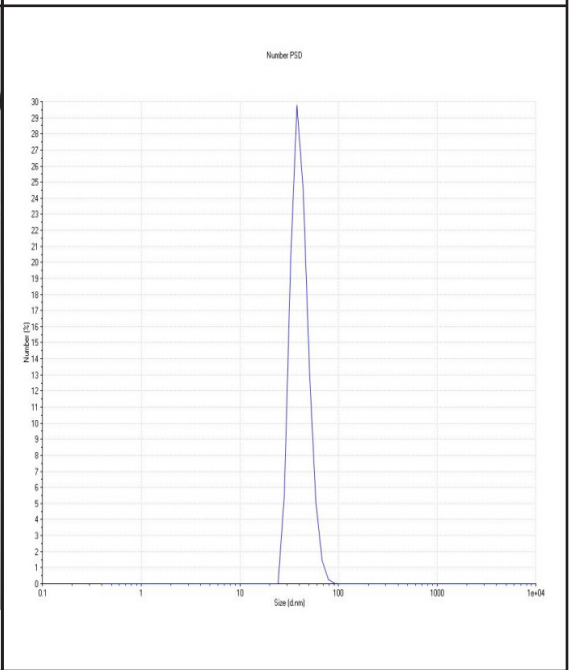
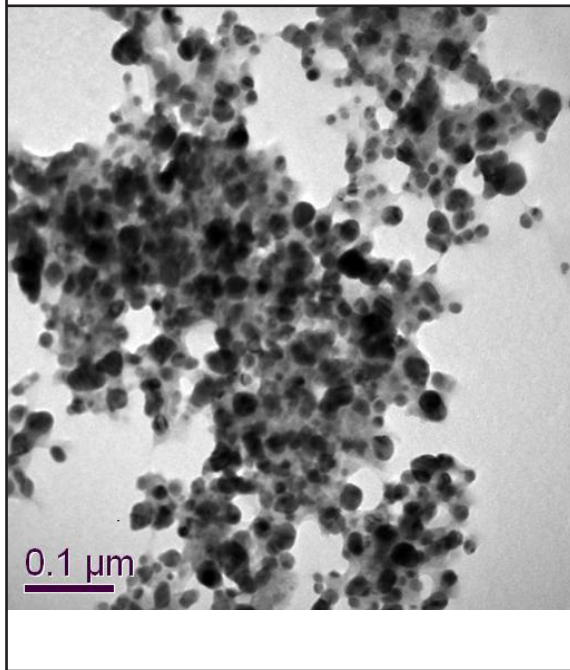
5.3.3c Silver nanoparticles from leachate from roots grown in cadmium(Cd) solution

5.3.3d Particle size analysis



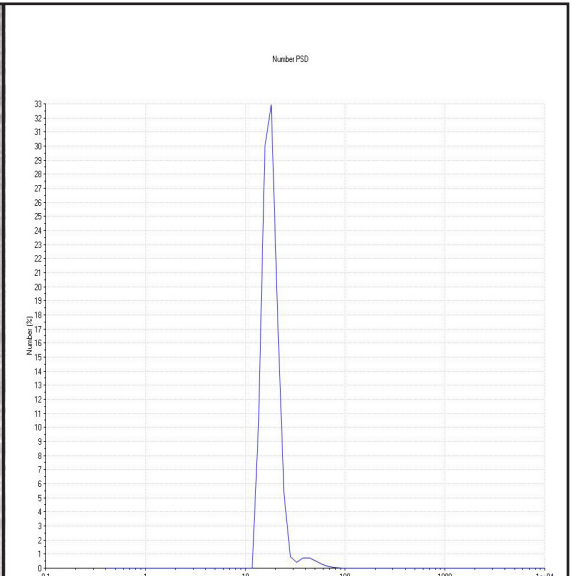
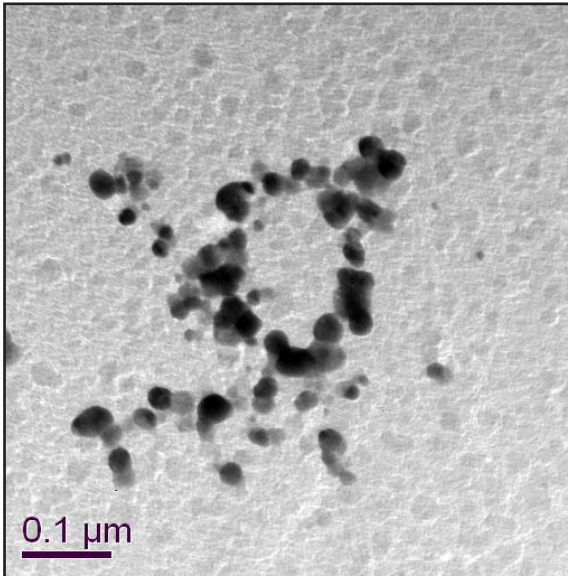
5.3.4a Silver nanoparticles from leachate from leaf grown in lead solution

5.3.4b Particle size analysis



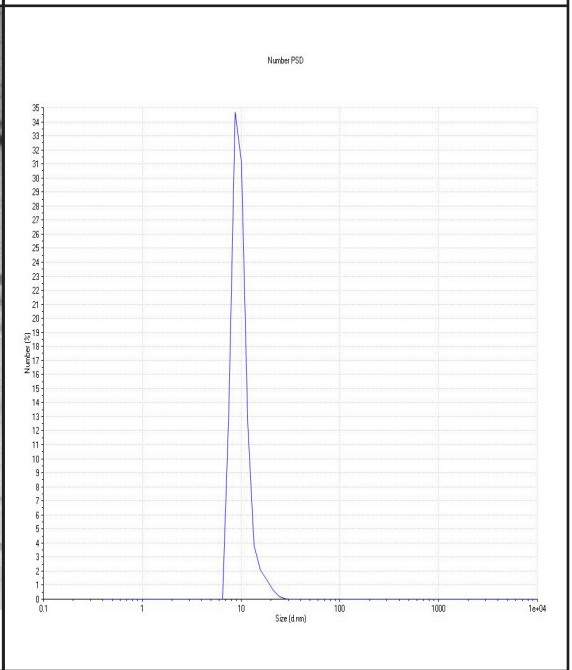
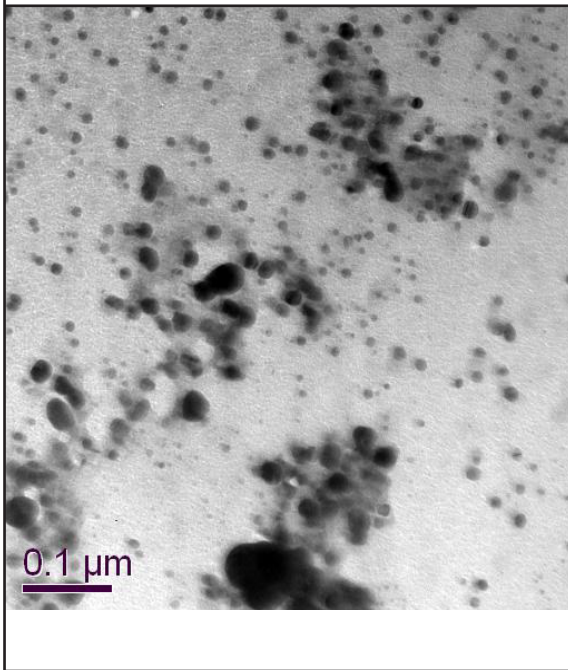
5.3.4c Silver nanoparticles from leachate from roots grown in lead solution

5.3.4d Particle size analysis



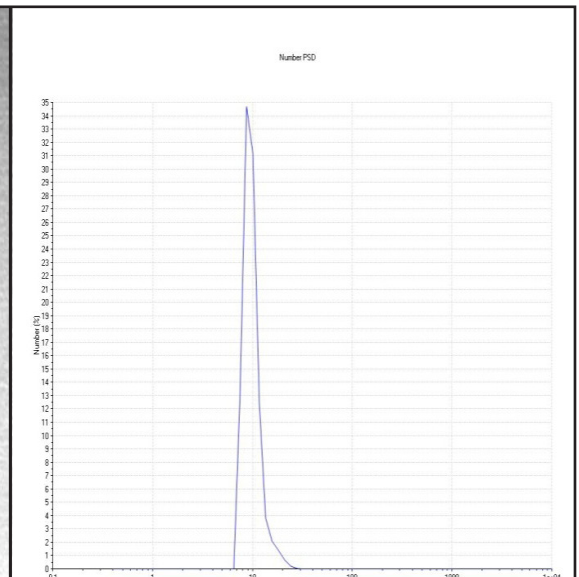
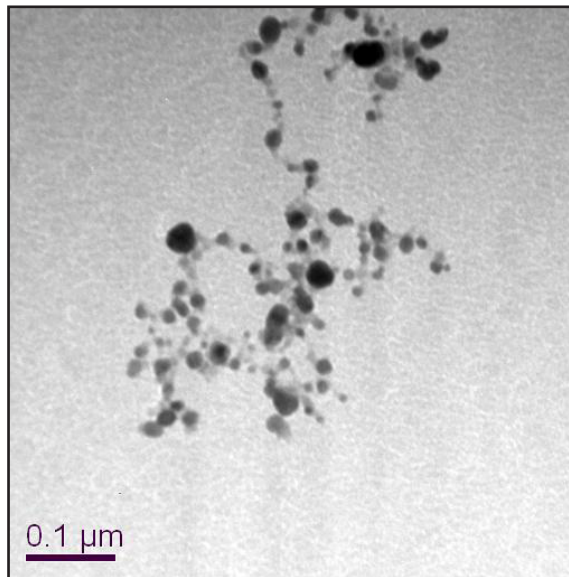
5.3.5a Silver nanoparticles from leachate from leave grown in Cu + Cr solution

5.3.5b Particle size analysis



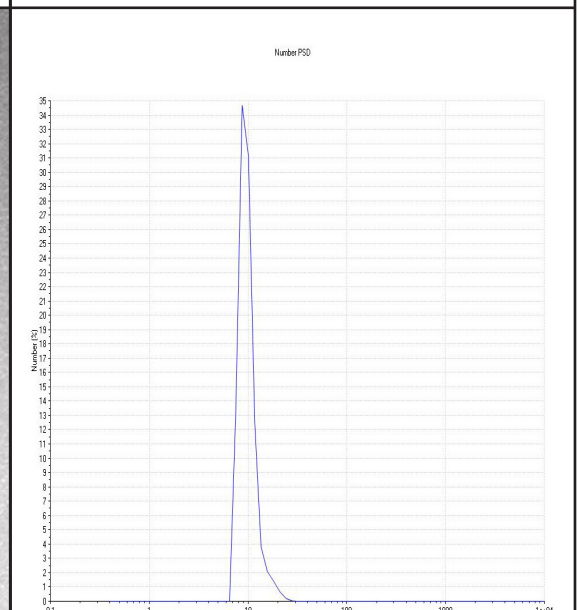
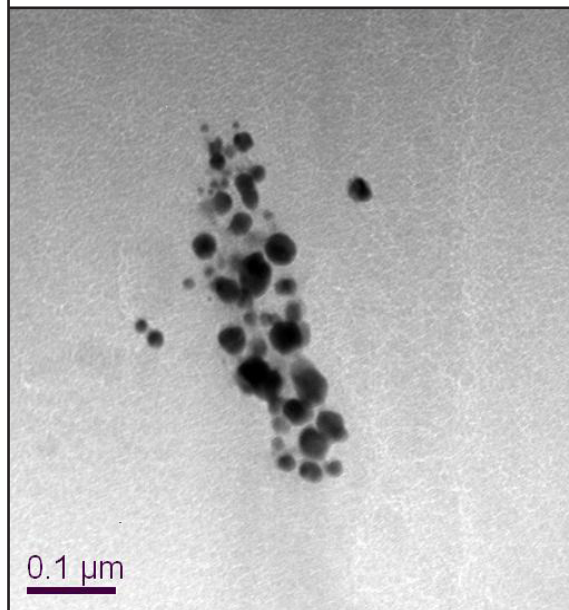
5.3.5c Silver nanoparticles from leachate from roots grown in Cu + Cr solution

5.3.5d Particle size analysis



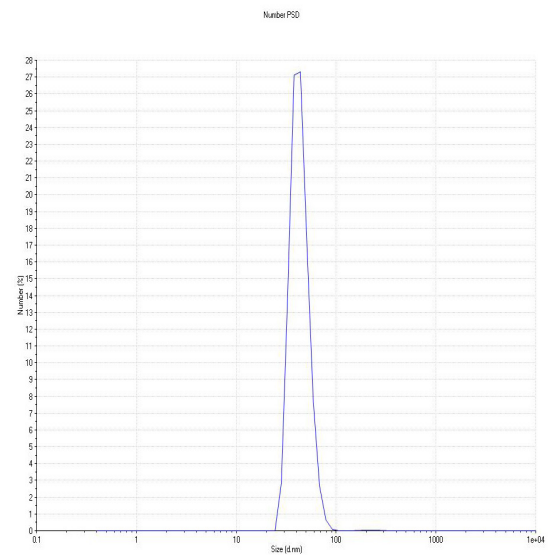
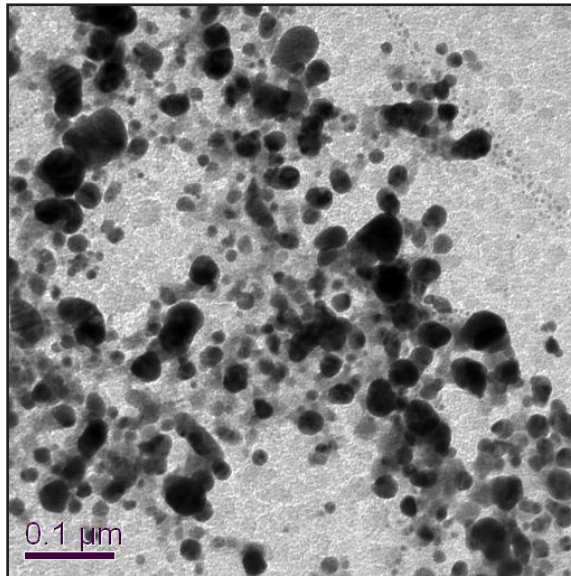
5.3.6a Silver nanoparticles from leachate from leaf grown in Cu + Cd solution

5.3.6b Particle size analysis



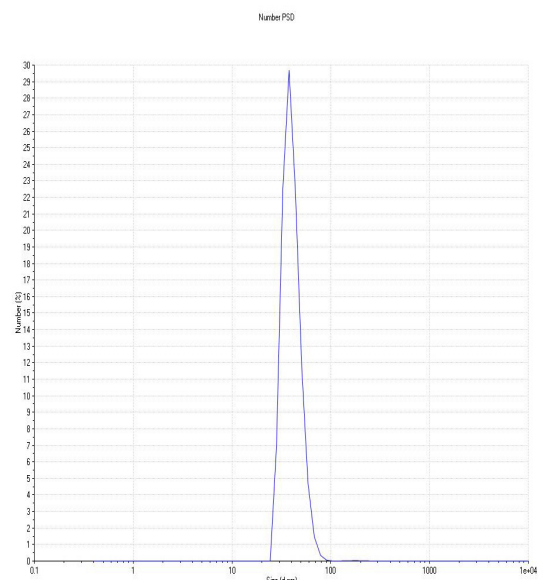
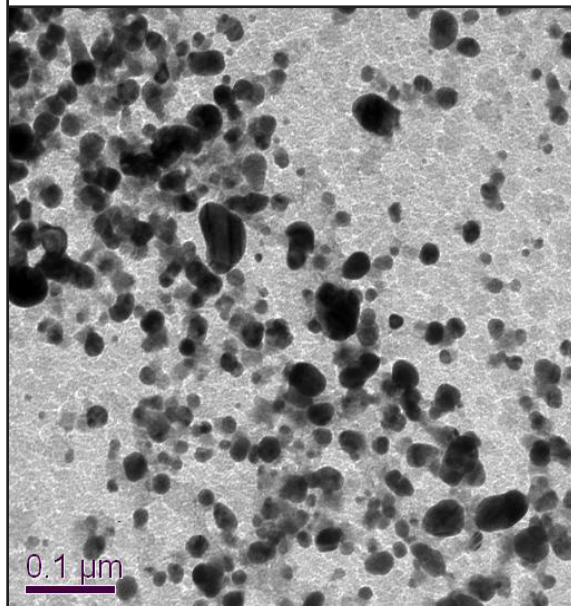
5.3.6c Silver nanoparticles from leachate from roots grown in Cu + Cd solution

5.3.6d Particle size analysis



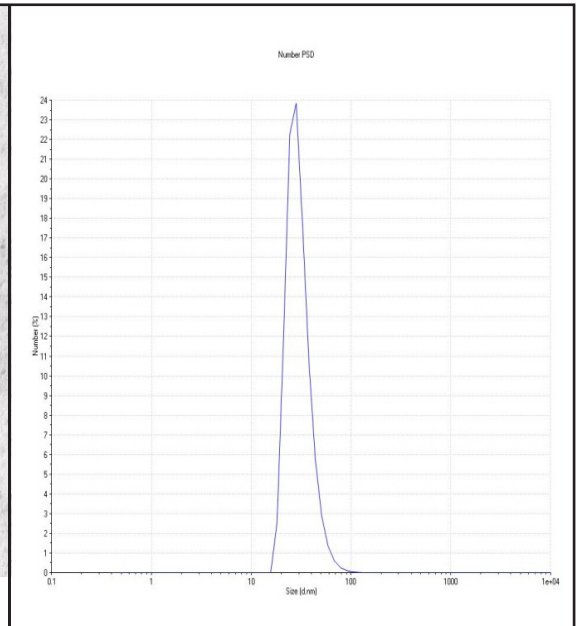
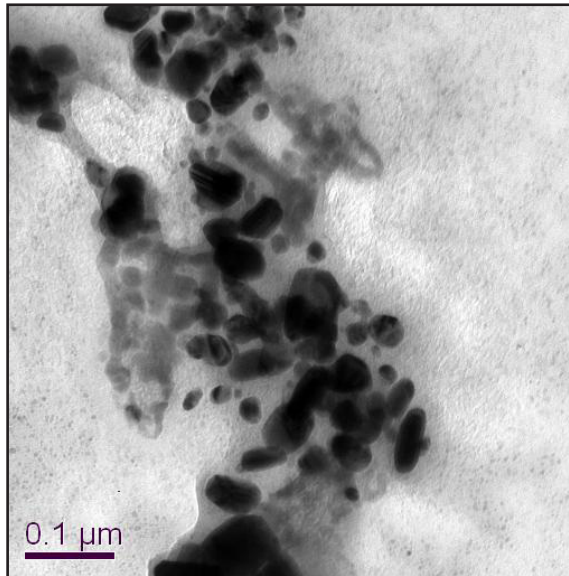
5.3.7a Silver nanoparticles from leachate from leave grown in Cu + Pb solution

5.3.7b Particle size analysis



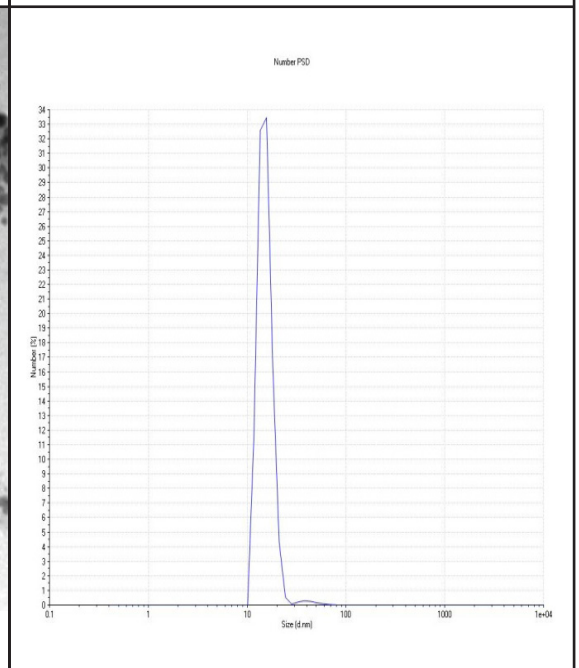
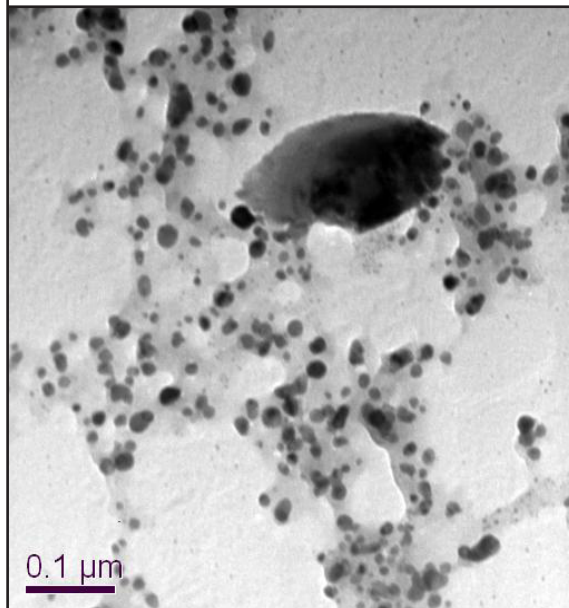
5.3.7c Silver nanoparticles from Leachate from roots grown in Cu + Pb solution

5.3.7d Particle size analysis



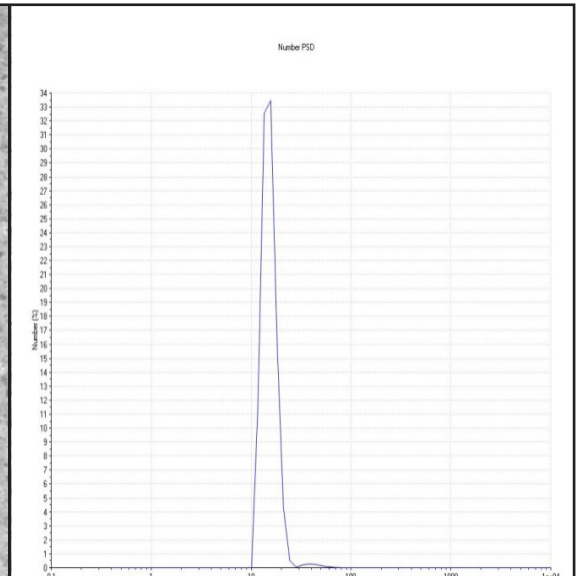
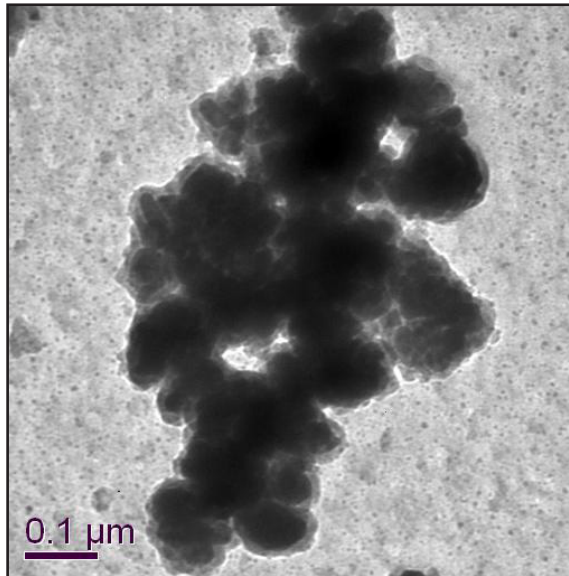
5.3.8a Silver nanoparticles from leachate from leave grown in Cr + Cd solution

5.3.8b Particle size analysis



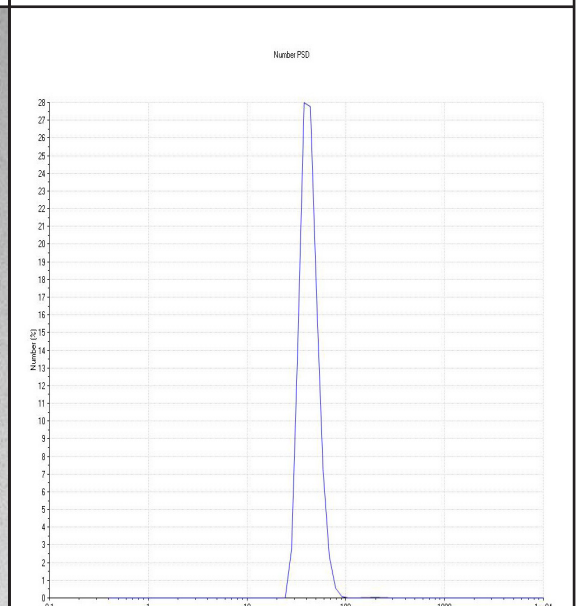
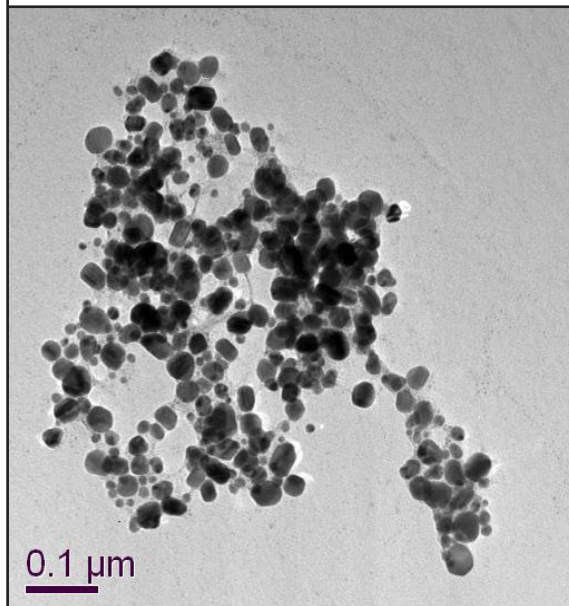
5.3.8c Silver nanoparticles from leachate from roots grown in Cr + Cd solution

5.3.8d Particle size analysis



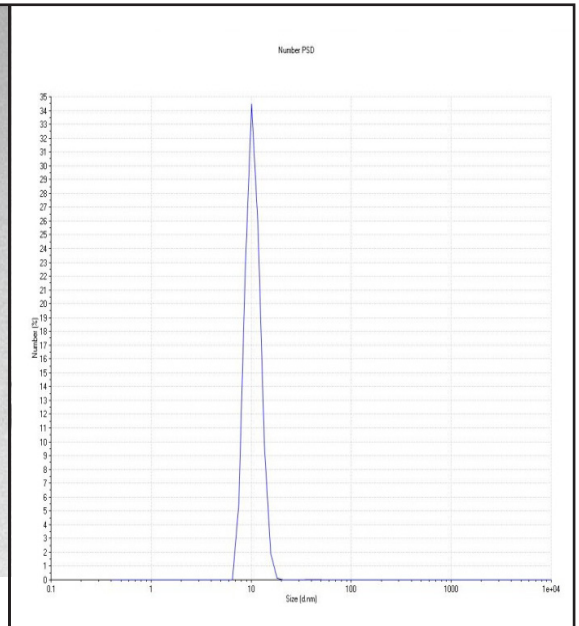
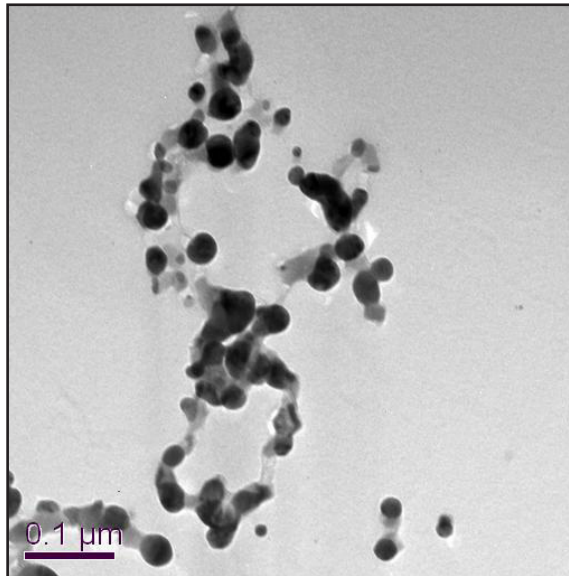
5.3.9a Silver nanoparticles from leachate from leave grown in Cr + Pb solution

5.3.9b Particle size analysis



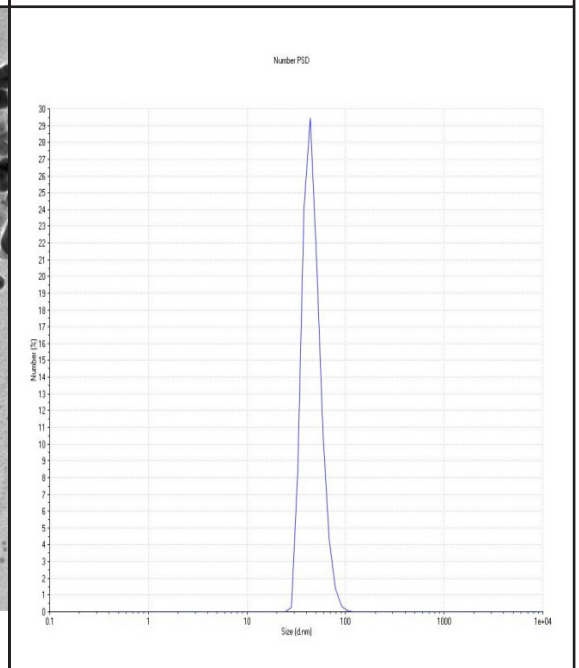
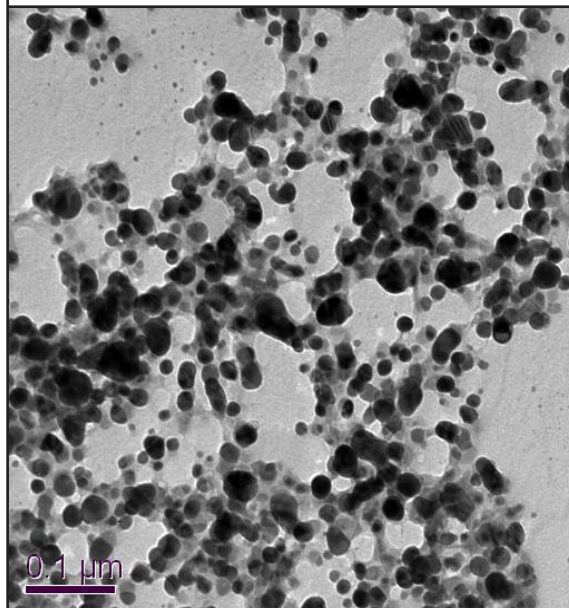
5.3.9c Silver nanoparticles from leachate from roots grown in Cr + Pb solution

5.3.9d Particle size analysis



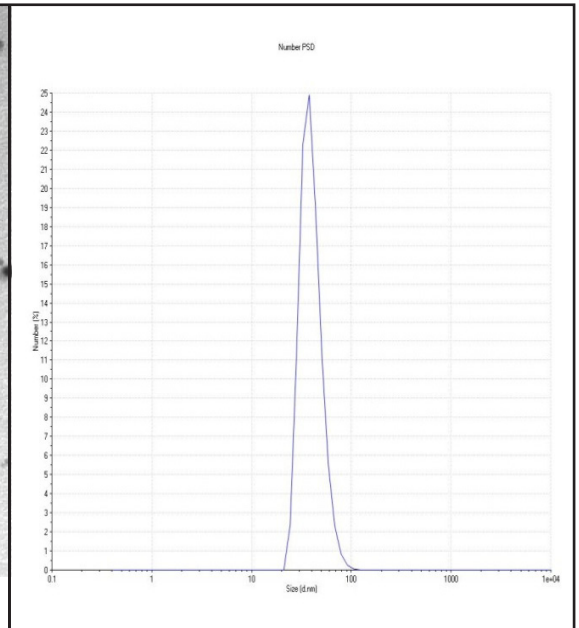
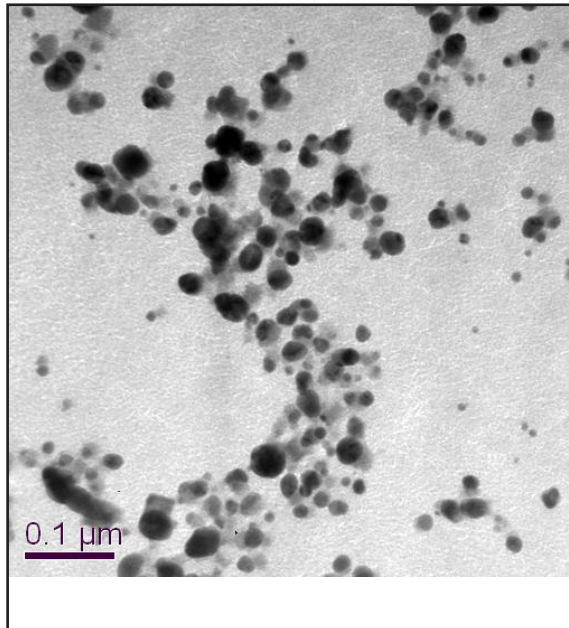
5.3.10a Silver nanoparticles from leachate from leave grown in Cd + Pb solution

5.3.10b Particle size analysis



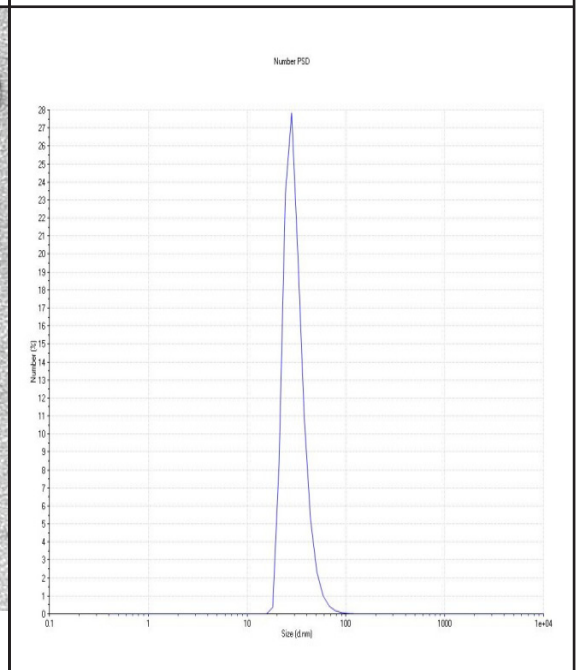
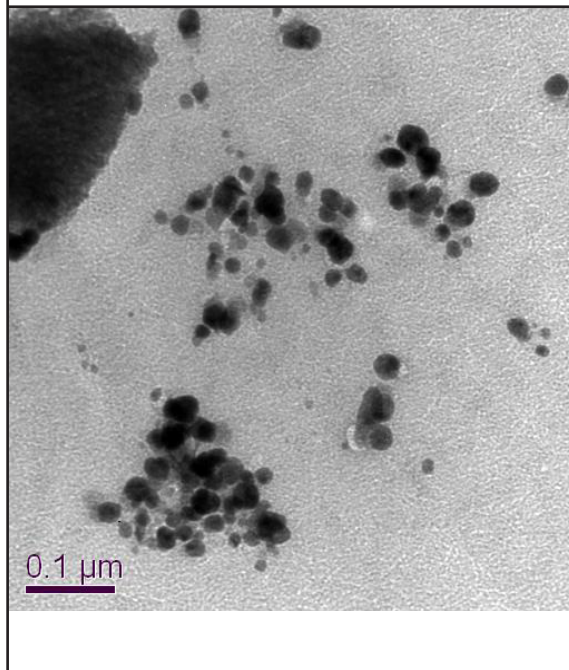
5.3.10c Silver nanoparticles from leachate from roots grown in Cd + Pb solution

5.3.10d Particle size analysis



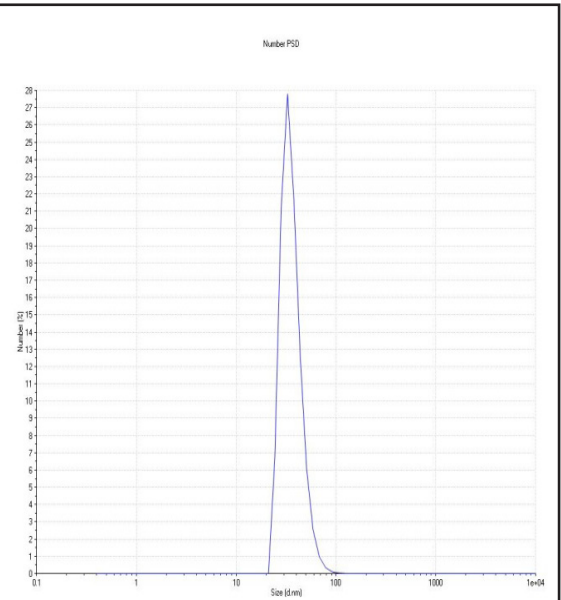
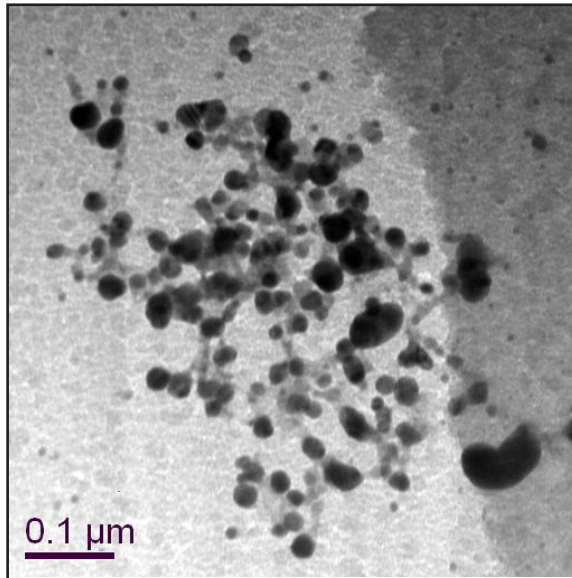
5.3.11a Silver nanoparticles from leachate from leaf grown in Cu + Cr + Cd solution

5.3.11b Particle size analysis



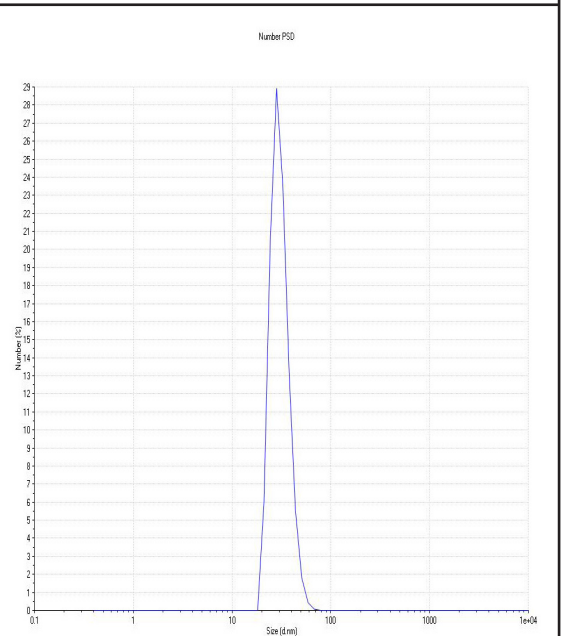
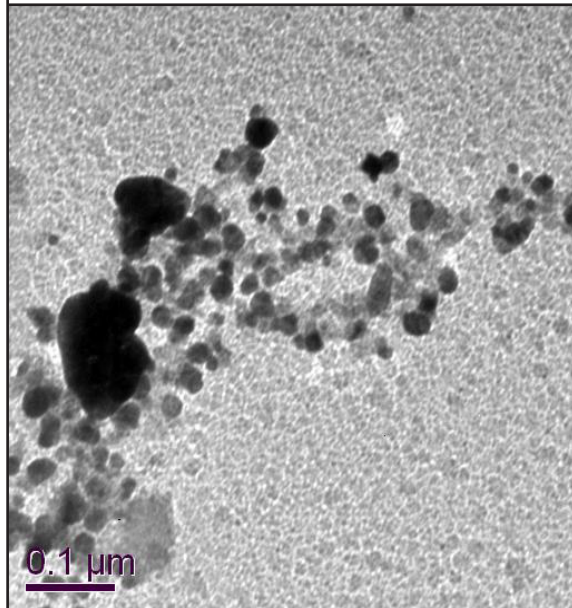
5.3.11c Silver nanoparticles from leachate from roots grown in Cu + Cr +Cd solution

5.3.11d Particle size analysis



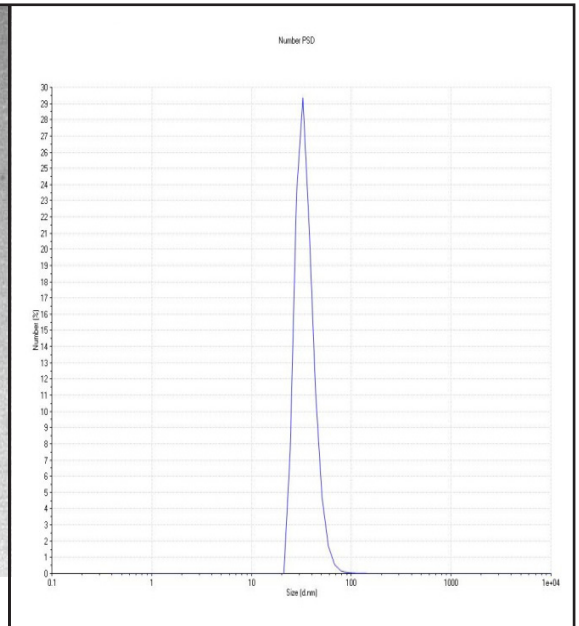
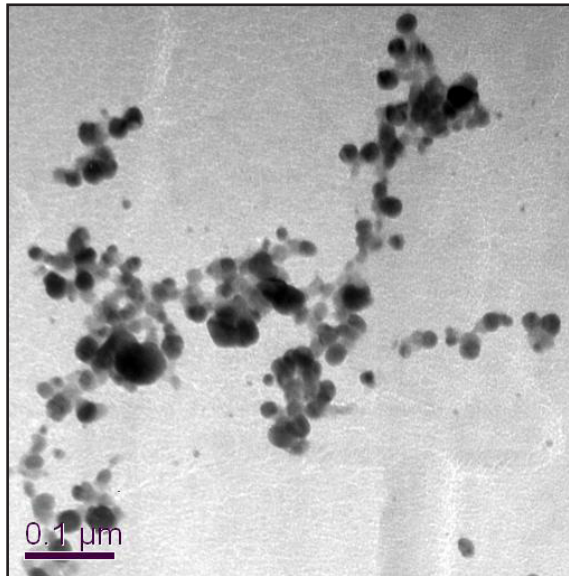
5.3.12a Silver nanoparticles from leachate from leaf grown in Cu + Cr + Pb solution

5.3.12b Particle size analysis



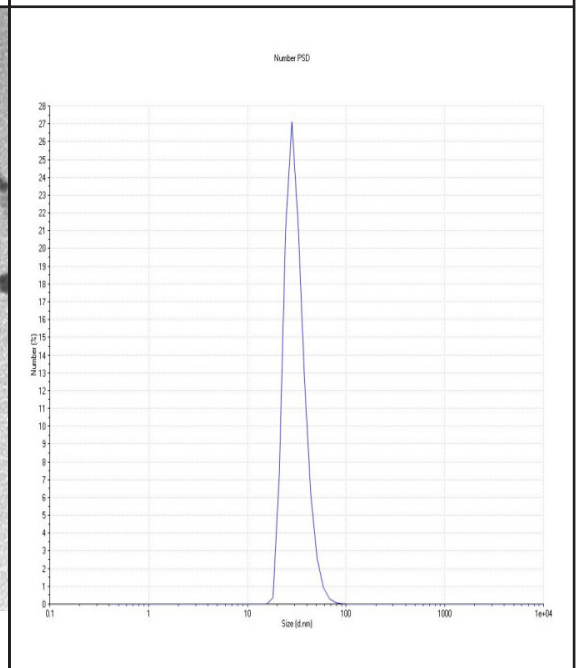
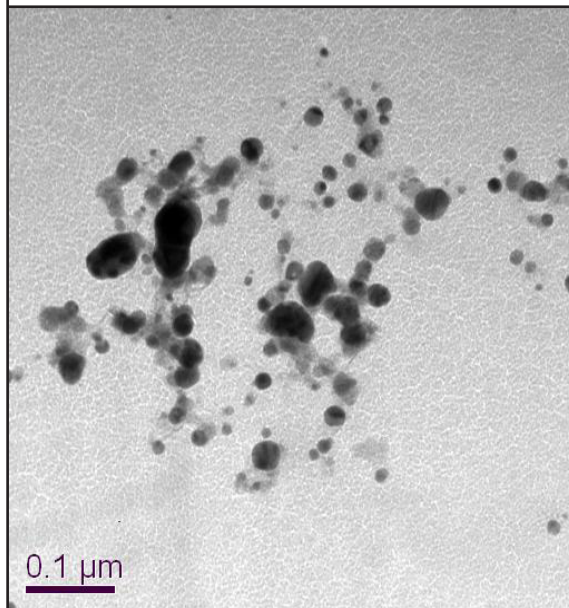
5.3.12c Silver nanoparticles from leachate from roots grown in Cu + Cr + Pb solution

5.3.12d Particle size analysis



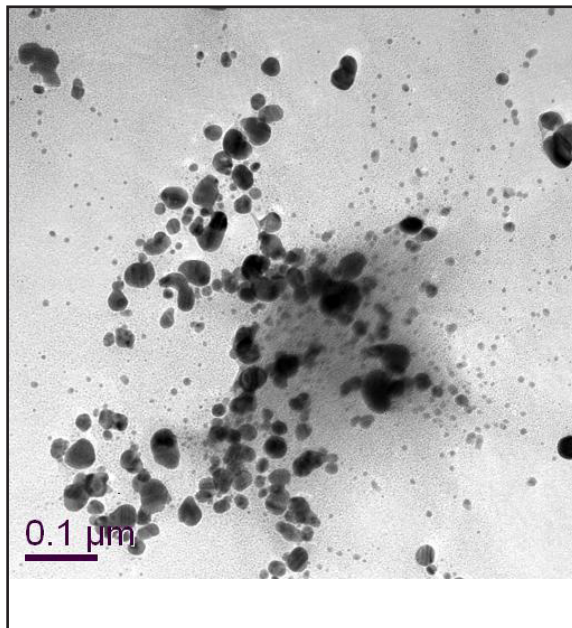
5.3.13a Silver nanoparticles from leachate from leave grown in Cu + Cd + Pb solution

5.3.13b Particle size analysis

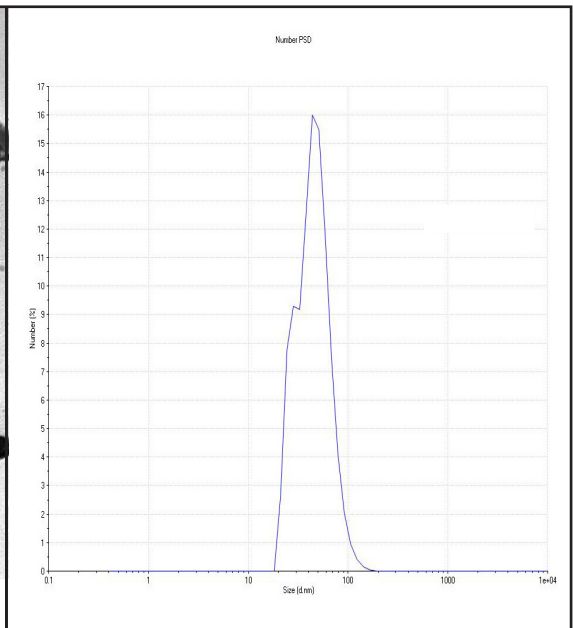


5.3.13c Silver nanoparticles from leachate from roots grown in Cu + Cd + Pb solution

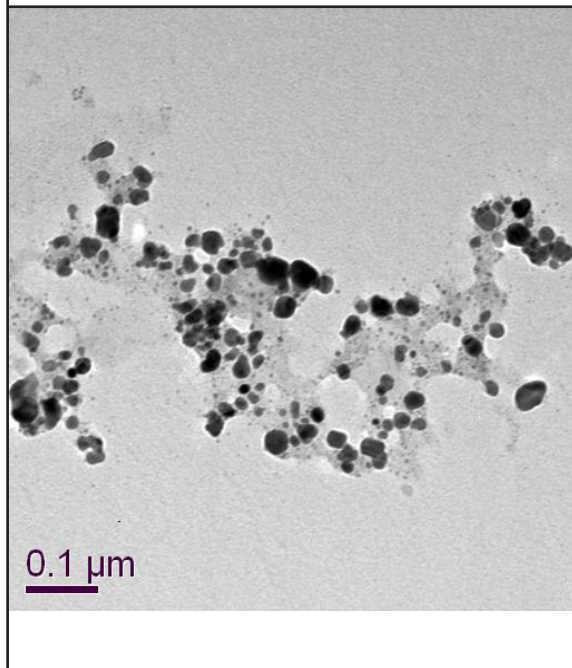
5.3.13d Particle size analysis



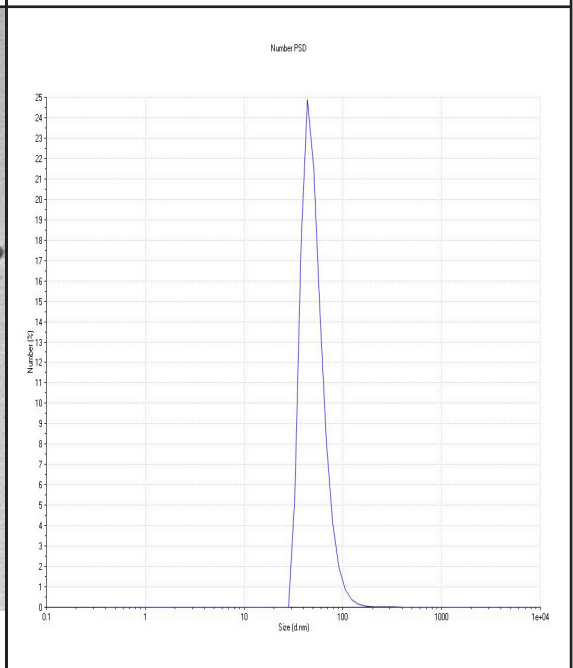
5.3.14a Silver nanoparticles from leachate from leaf grown in Cr + Cd + Pb solution



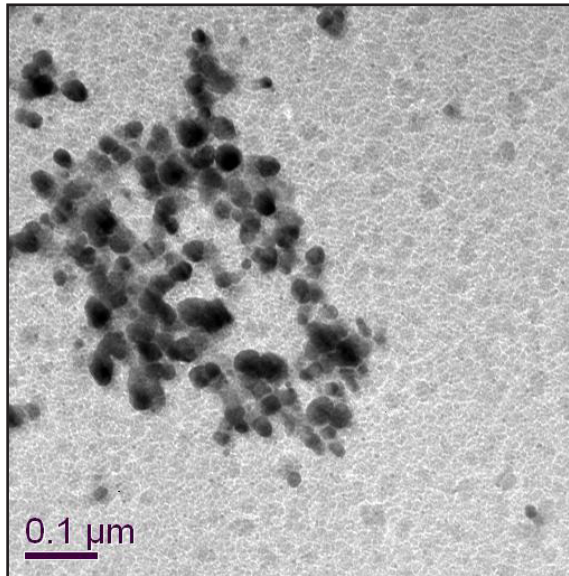
5.3.14b Particle size analysis



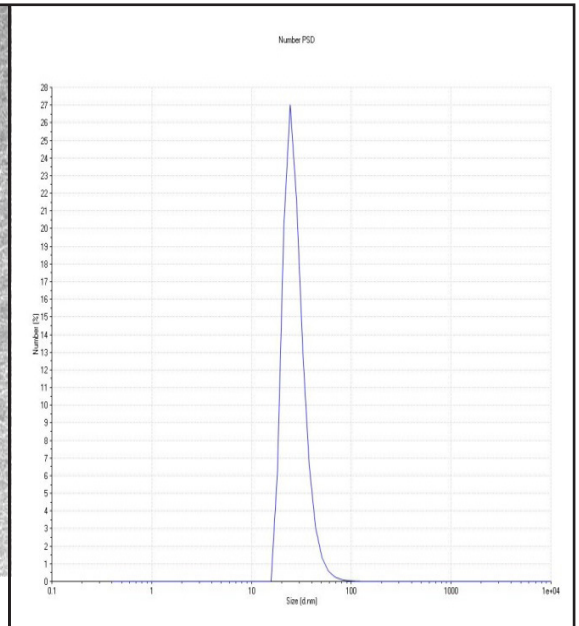
5.3.14c Silver nanoparticles from leachate from roots grown in Cr + Cd + Pb solution



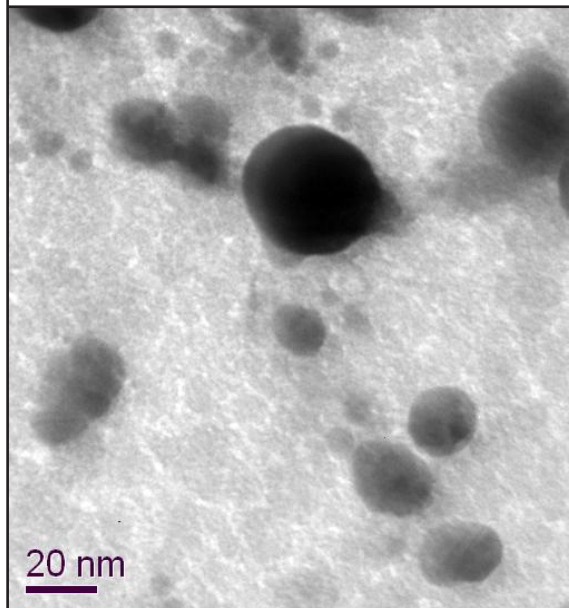
5.3.14d Particle size analysis



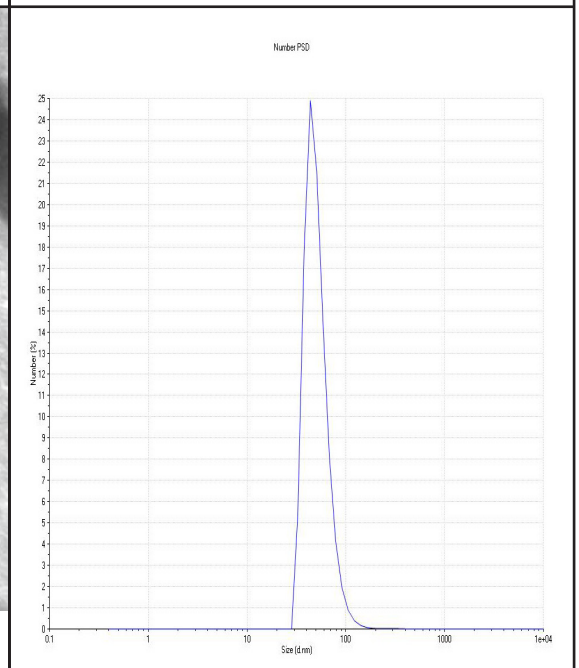
5.3.15a Silver nanoparticles from leachate from leaf grown in all metals solution



5.3.15b Particle size analysis



5.3.15c Silver nanoparticles from leachate from roots grown in all metals solution



5.3.15d Particle size analysis

Effect of metal ions and their combinations on the plant's potential to synthesize Silver nanoparticles:

Serial #	Particles	Average size (nm)
1	Copper (Cu)	115.6*
2	Chromium (Cr)	44.58
3	Cadmium (Cd)	66.77
4	Lead (Pb)	96.26

Table 5.3.1, 2, 3 & 4b Comparison of particle size analysis of leave with single metal

Serial #	Particles	Average size (nm)
1	Copper (Cu)	57.47
2	Chromium (Cr)	118.40*
3	Cadmium (Cd)	55.00
4	Lead (Pb)	81.43

Table 5.3.1, 2, 3&4d Comparison of particle size analysis of roots with single metal

The results show that the average particle size for copper in leaves is larger than for any other single metal but for roots the largest particle size was found in plants grown in chromium solution. However* for the copper leaves and chromium roots, the zeta sizing results are biased towards a larger particle diameter due to possible aggregation of the particles. The true means is likely to be nearer to 50nm which represents individual particle diameters when the TEM micrographs are examined..

Average size when two elements were combined:

Serial #	Particles	Average size (nm)
1	Cu-Cr	60.09
2	Cu-Cd	81.43
3	Cu-Pb	73.63
4	Cr-Cd	52.38
5	Cr-Pb	54.67
6	Cd-Pb	75.9

Table 5.3.5, 6, 7, 8, 9&10b Comparison of particle size analysis of leave with two metals

Serial #	Particles	Average size (nm)
1	Cu-Cr	64.79
2	Cu-Cd	54.67
3	Cu-Pb	57.95
4	Cr-Cd	61.13
5	Cr-Pb	72.79
6	Cd-Pb	64.49

Table 5.3.5, 6, 7, 8, 9& 10d Comparison of particle size analysis of roots with two metals

Six trials were performed by using combination of two elements and effect of combination on average size of nanoparticales produced from leaf and root extracts was determined. Results are given in figures 5.3.5, 6, 7, 8, 9, 10b and d for leaves and roots respectively. In leaf extracts maximum size (81.43nm) was observed for Cu-Cd pair and for roots it was higher (72.79nm) for Cr-Pb pair.

Comparison of Average size when three elements were combined:

Serial #	Particles	Average size (nm)
1	Cu-Cd-Cr	51.49
2	Cu-Cr-Pb	54.88
3	Cu-Cd-Pb	64.79
4	Cd-Cr-Pb	98.62

Table 5.3.11, 12, 13 & 14b Comparison of particle size analysis of leave with three metals

Serial #	Particles	Average size (nm)
1	Cu-Cd-Cr	70.58
2	Cu-Cr-Pb	71.27
3	Cu-Cd-Pb	64.79
4	Cd-Cr-Pb	89.61

Table 5.3.11, 12, 13 & 14d Comparison of particle size analysis of roots with three metals

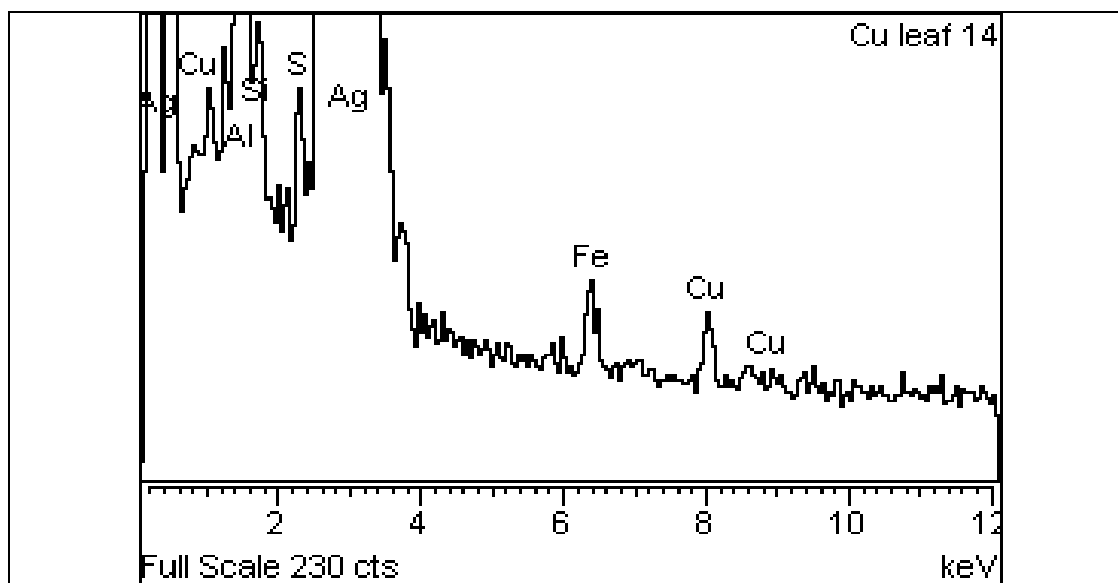
Four trials for the synthesis of nanoparticles, from leaf and root extracts were performed by using combination of three elements. Average size for each combination is given in figures 5.3.11, 12, 13, 14b and d for leaf and root extracts, respectively. Higher size was observed for Cd-Cr-Pb combinations for both leaf and root extracts with the values of 98.62nm and 89.61nm, respectively.

Average size when four elements were combined:

Results in figures 5.3.15b and d show effect of combination of all the four elements (Cu-Cd-Cr-Pb) on the size of nanoparticles produced from leaf and root extracts. Average size was found to be 52.48nm and 46.93 for leaf and root extracts, respectively.

5.4 Possible incorporation of metals into nanoparticles

Leachates obtained by boiling the biomass accumulated during the hydroponic experiments, were mixed with silver nitrate to see whether any of the accumulated metals would be extracted and combined within the manufactured silver nanoparticles. The analysis was carried out by transmission electron microscopy (TEM) and energy dispersive X-ray analysis (EDX). The results show that Cu is the only metal which combined with the silver nanoparticle. X-ray spectra acquired from the silver nanoparticles are shown in Figs 5.4.1 to 5.4.15.



5.4.1.a X-ray spectrum from silver nanoparticles produced from leaf extract containing copper

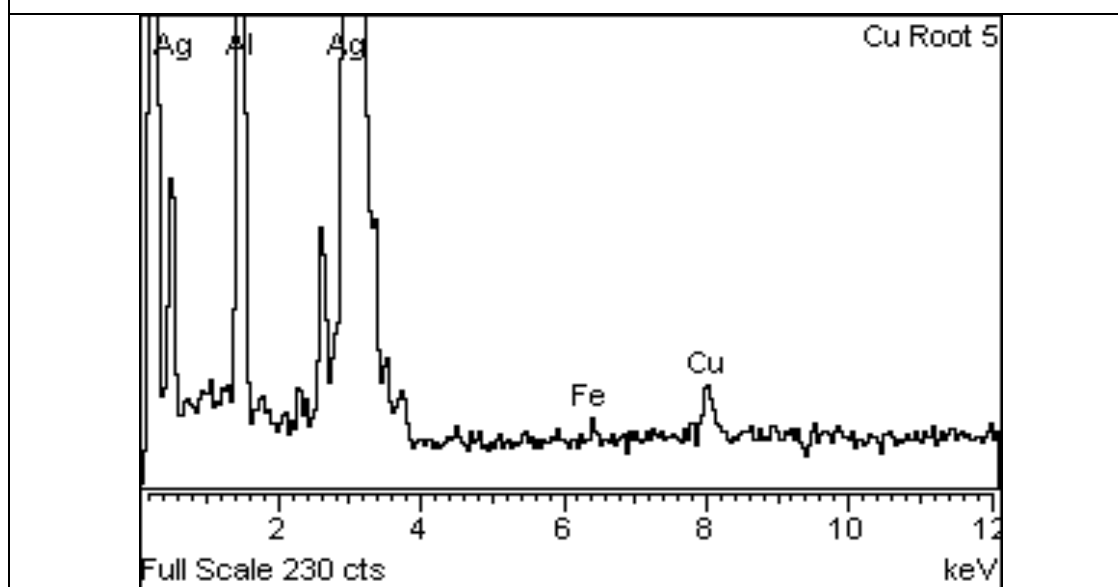
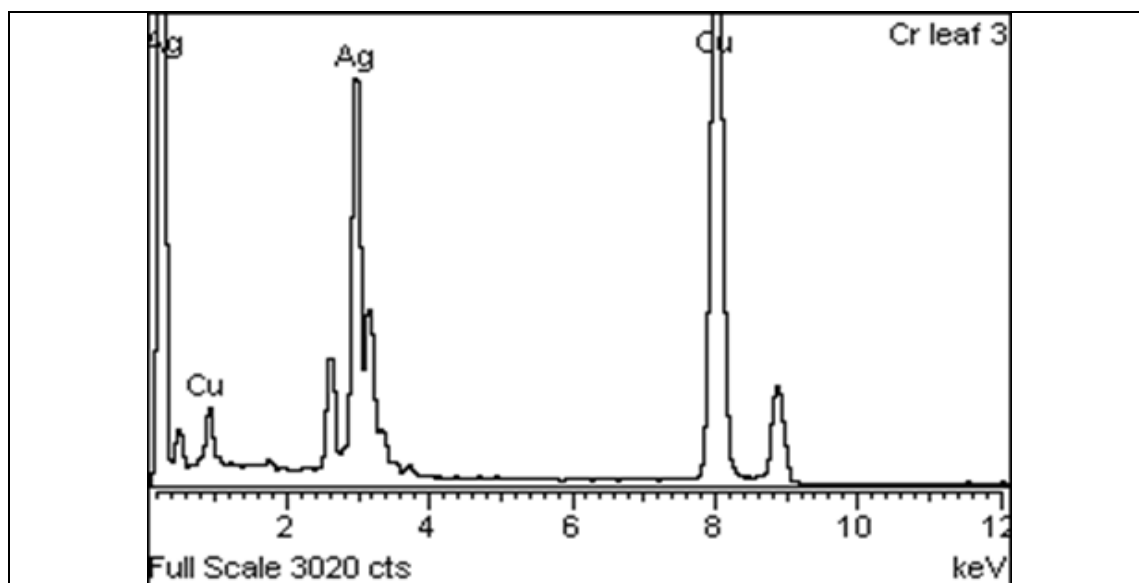
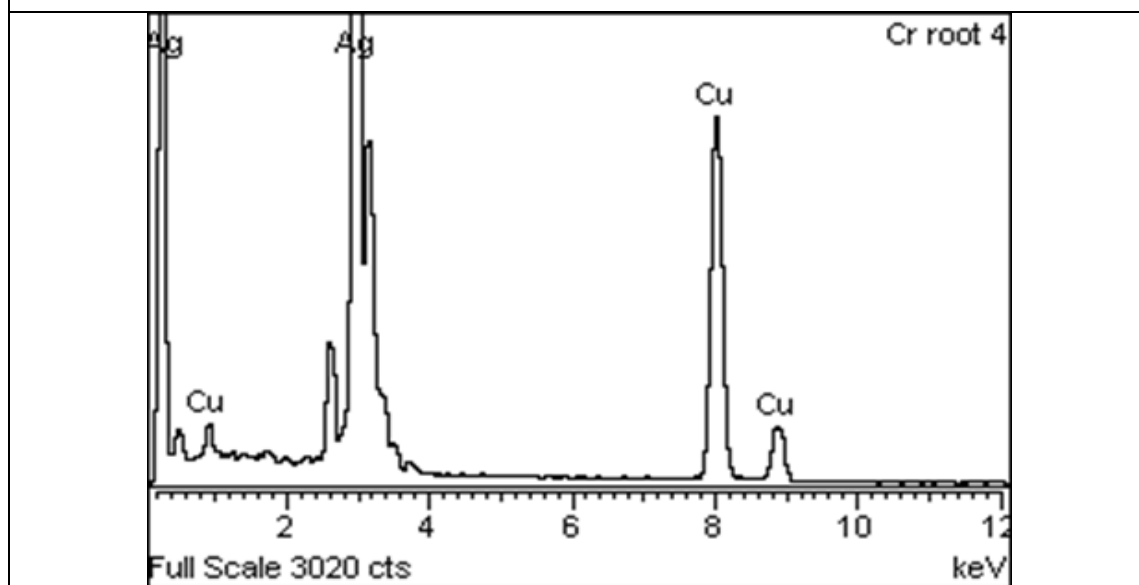


Fig 5.4.1.b X-ray spectrum from silver nanoparticles produced from root extract containing copper

The spectra show a copper peak at 8.04keV and a silver peak at 2.98keV, the samples were mounted onto aluminium microscope grids coated with an evaporated carbon film.

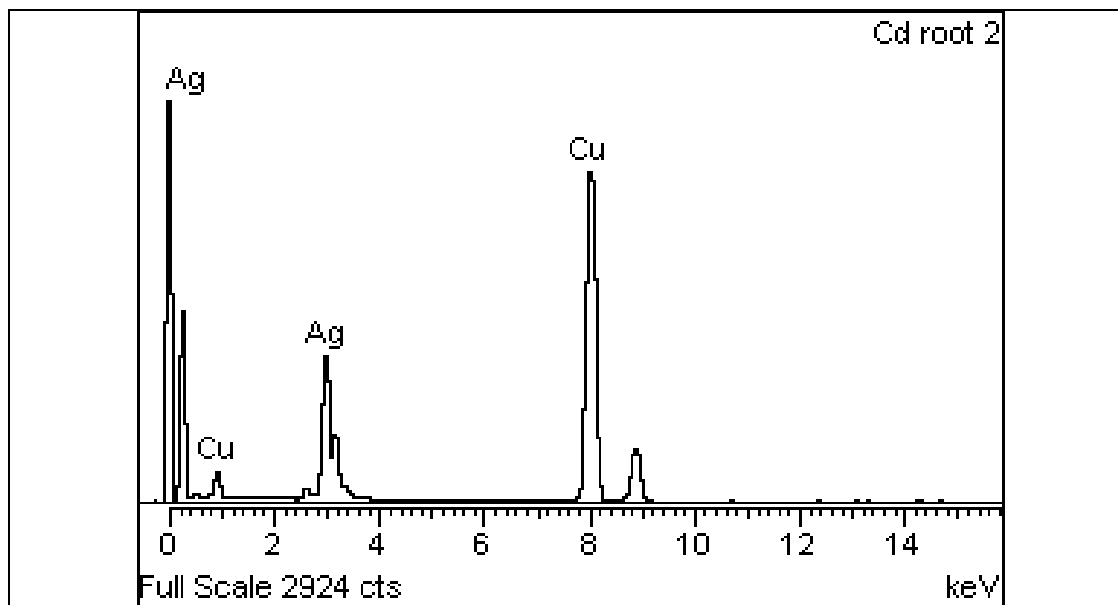


5.4.2.a X-ray spectrum from silver nanoparticles produced from leaf extract containing chromium

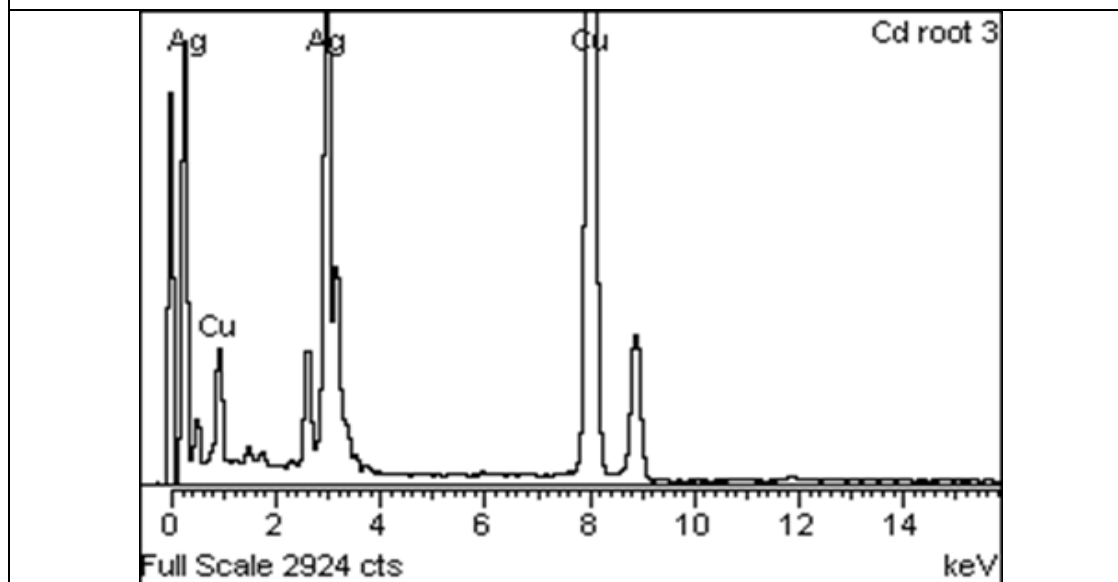


5.4.2.b X-ray spectrum from silver nanoparticles produced from root extract containing chromium

The spectra do not show a chromium peak at 5.41keV, a silver peak at 2.98keV is present; the samples were mounted onto copper microscope grids coated with an evaporated carbon film.



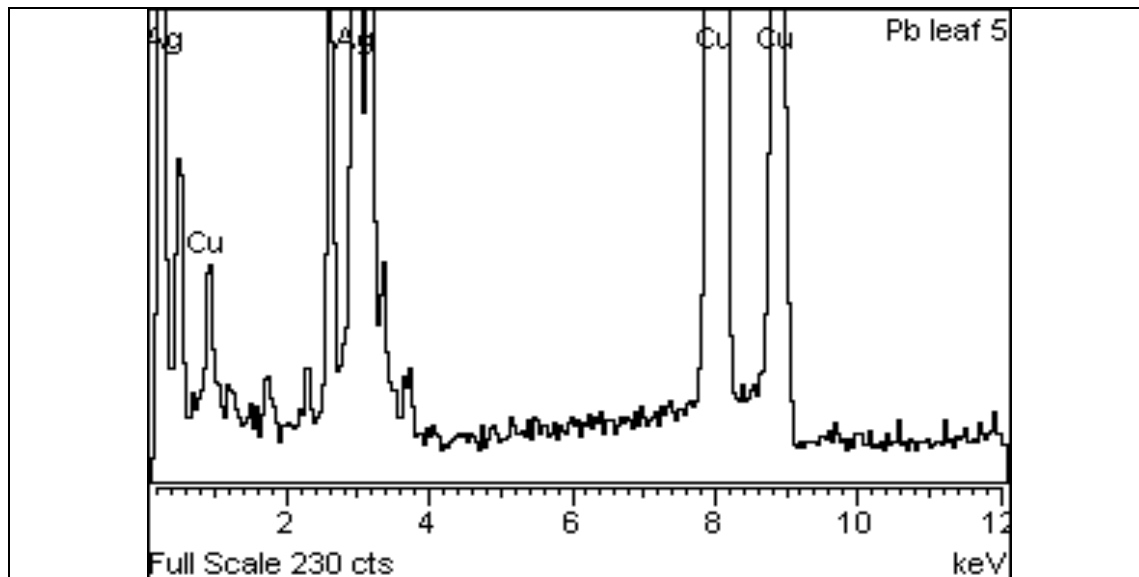
5.4.3.a X-ray spectrum from silver nanoparticles produced from leaf extract containing cadmium



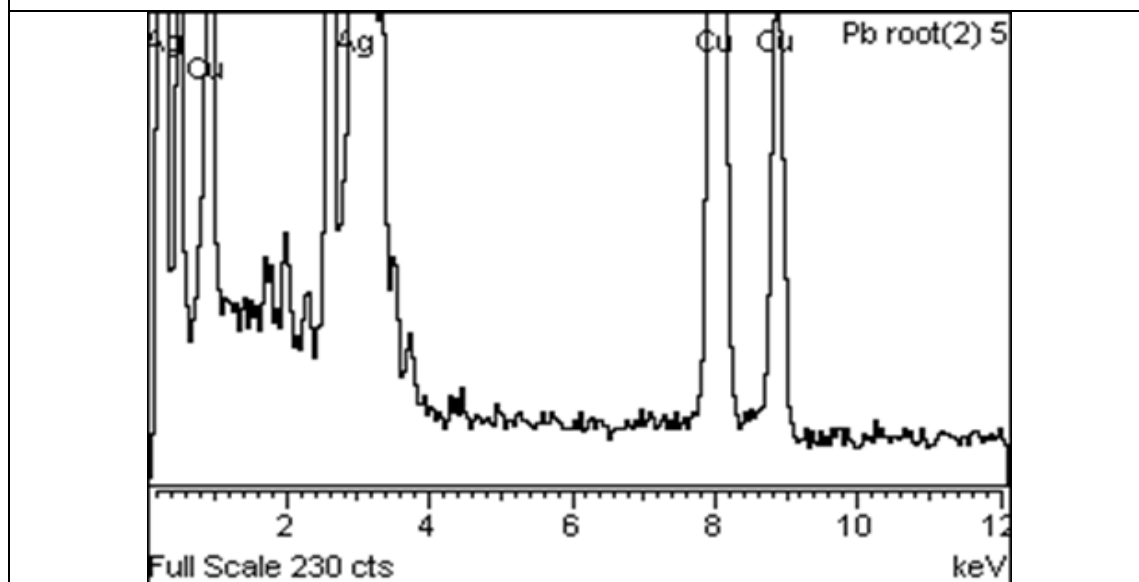
5.4.3.b X-ray spectrum from silver nanoparticles produced from root extract containing cadmium

The spectra do not show a cadmium peak at 3.13keV, a silver peak at 2.98keV is present; the samples were mounted onto copper microscope grids coated with an evaporated carbon film. The silver and cadmium L peaks overlap therefore the silver

may mask the presence of cadmium. The higher energy Cd line (K alpha) cannot be detected using a silicon drift detector at 100kV.

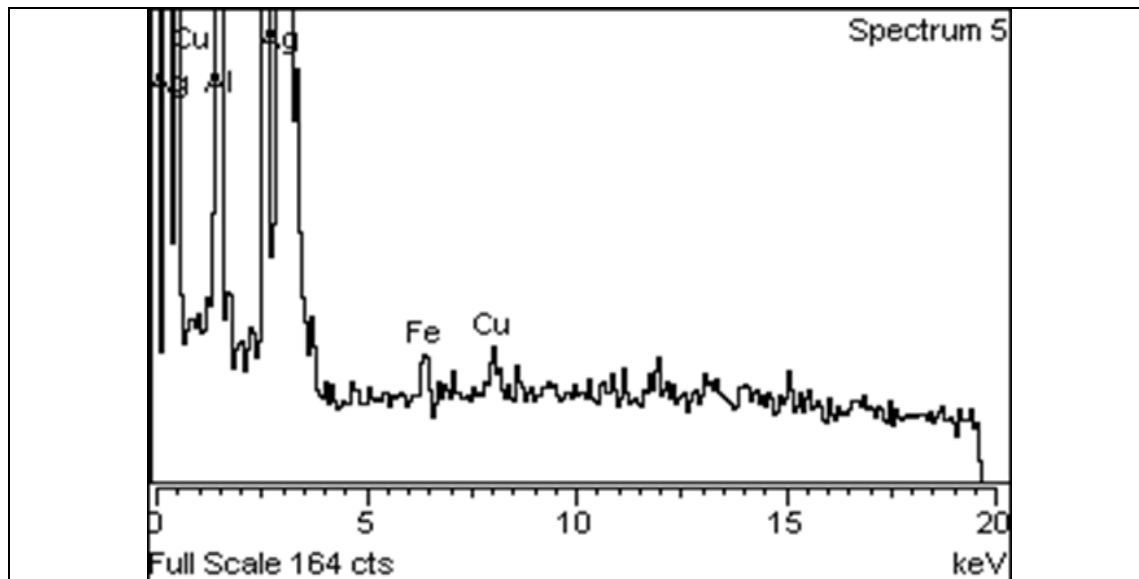


5.4.4.a X-ray spectrum from silver nanoparticles produced from leaf extract containing lead

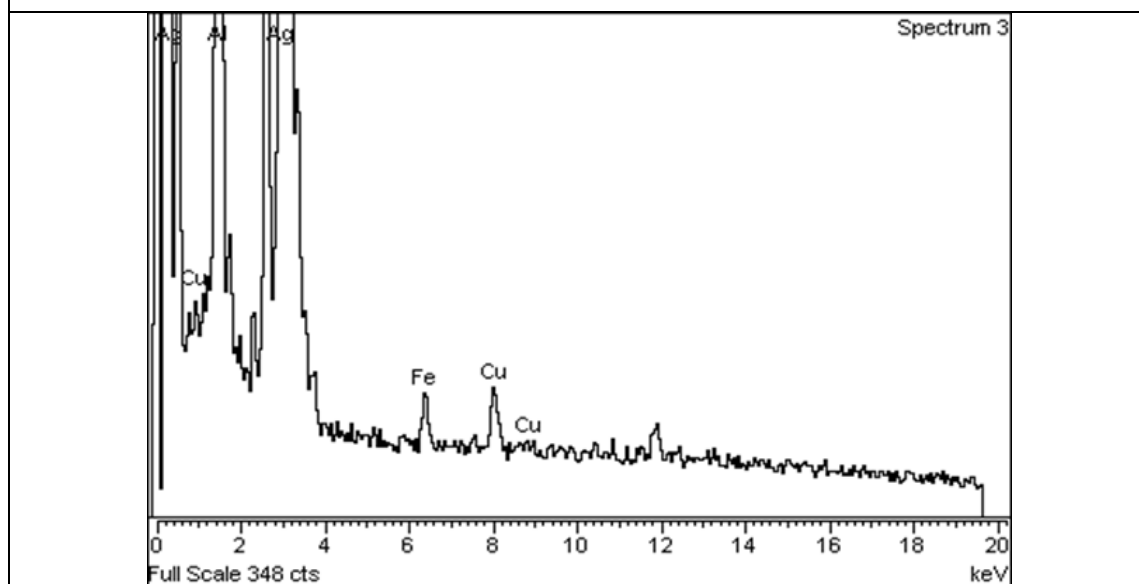


5.4.4.b X-ray spectrum from silver nanoparticles produced from root extract containing lead

The spectra do not show a lead peak at 2.34 or 10.55keV, a silver peak at 2.98keV is present; the samples were mounted onto copper microscope grids coated with an evaporated carbon film.

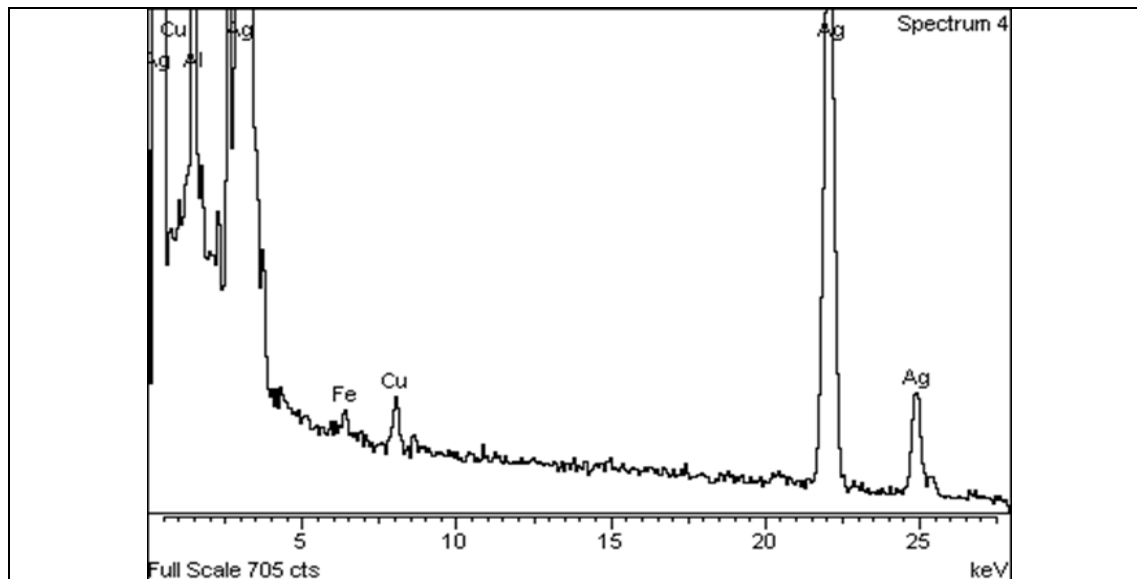


5.4.5.a X-ray spectrum from silver nanoparticles produced from leaf extract containing copper and chromium

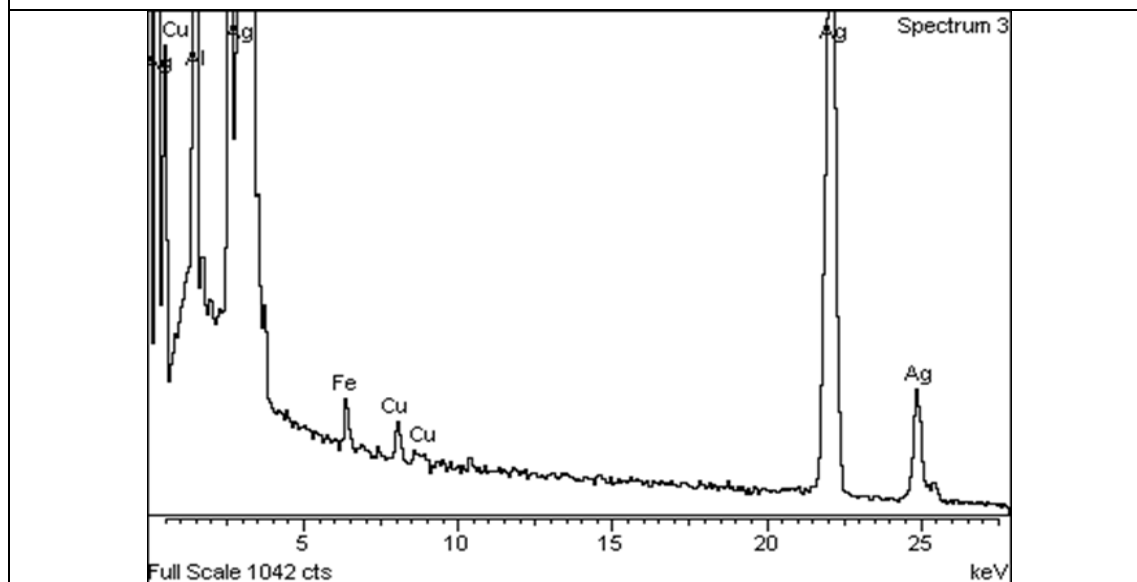


5.4.5.b X-ray spectrum from silver nanoparticles produced from root extract containing copper and chromium

The spectra show a copper peak at 8.04keV but a chromium peak at 5.44keV is not present, a silver peak at 2.98keV is present, the samples were mounted onto aluminium microscope grids coated with an evaporated carbon film.



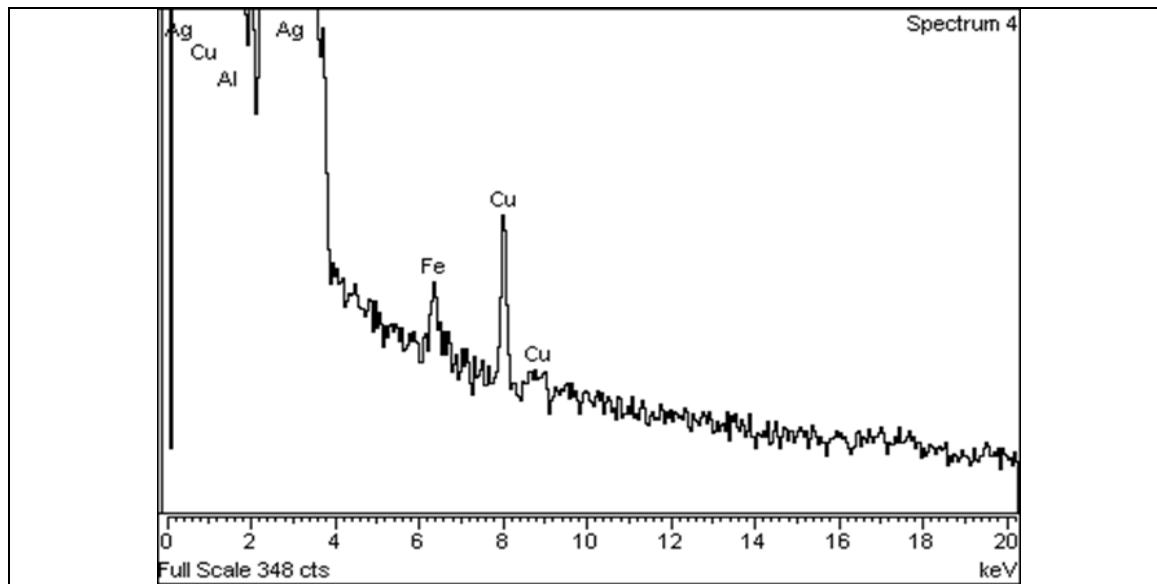
5.4.6.a X-ray spectrum from silver nanoparticles produced from leaf extract containing copper and cadmium



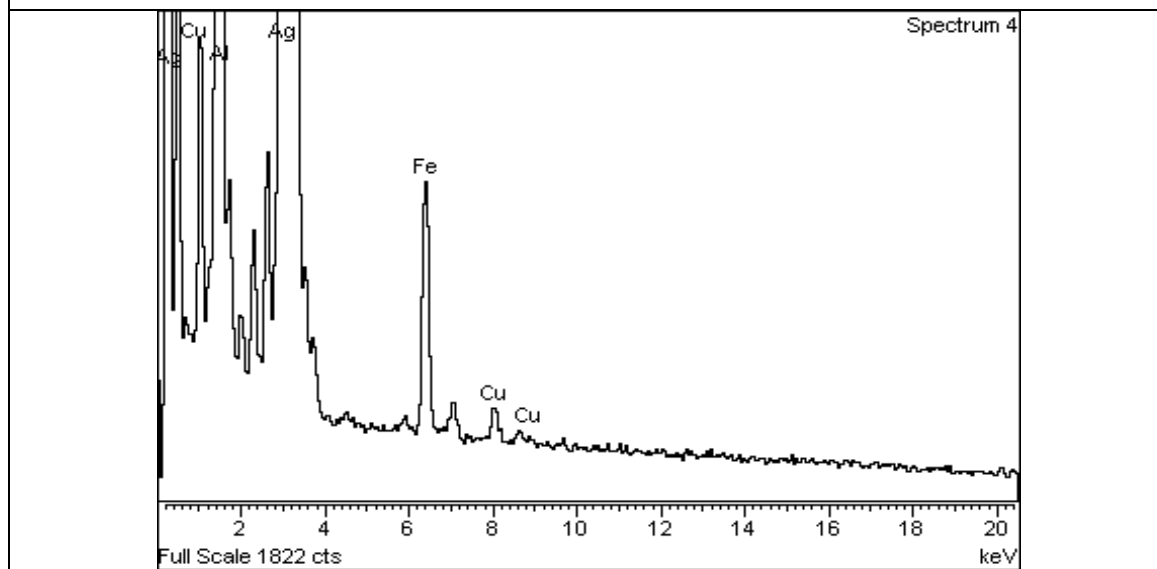
5.4.6.b X-ray spectrum from silver nanoparticles produced from root extract containing copper and cadmium

The spectra show a copper peak at 8.04keV but cadmium peaks at 3.13 and 23.17keV are not present, a silver peak at 2.98keV is present, the samples were mounted onto

aluminium microscope grids coated with an evaporated carbon film. The silver and cadmium K and L peaks overlap therefore the silver may mask the presence of cadmium.

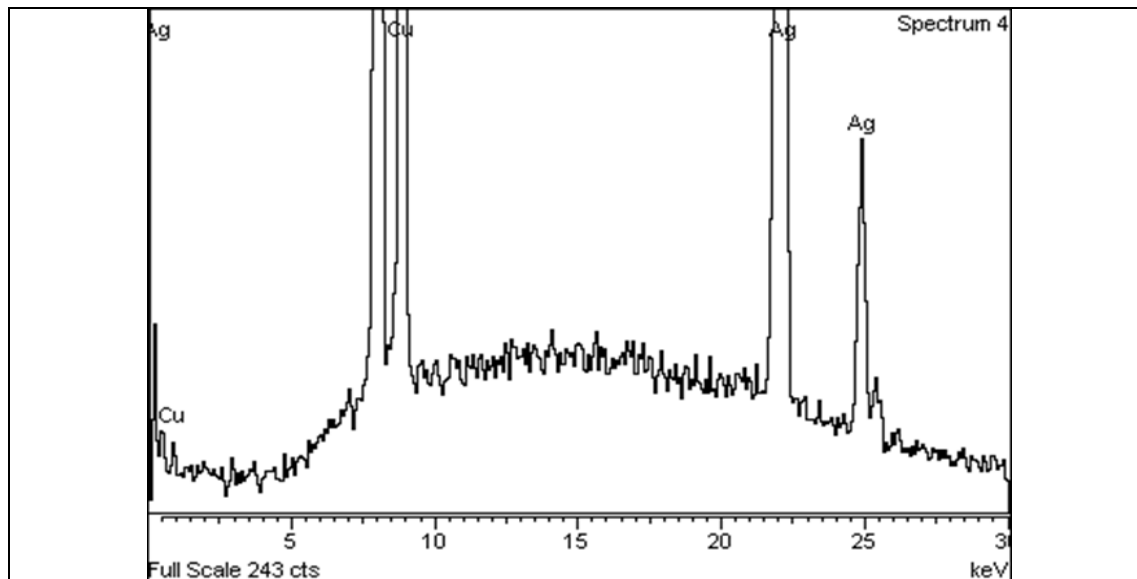


5.4.7.a X-ray spectrum from silver nanoparticles produced from leaf extract containing copper and lead

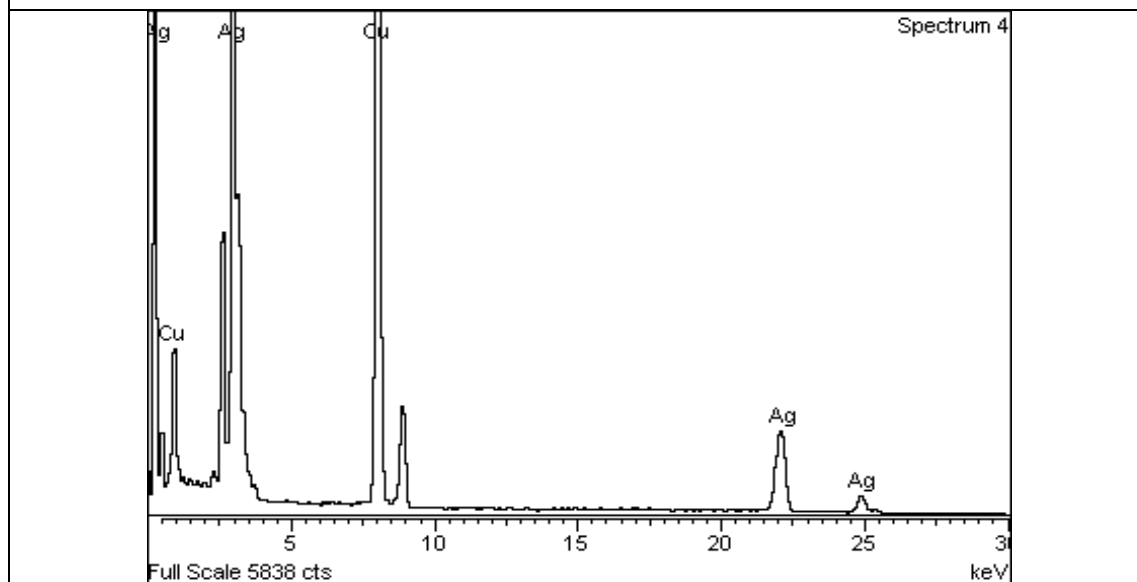


5.4.7.b X-ray spectrum from silver nanoparticles produced from root extract containing copper and lead

The spectra show a copper peak at 8.04keV but a lead peak at 10.55 keV is not present, a silver peak at 2.98keV is present, the samples were mounted onto aluminium microscope grids coated with an evaporated carbon film.



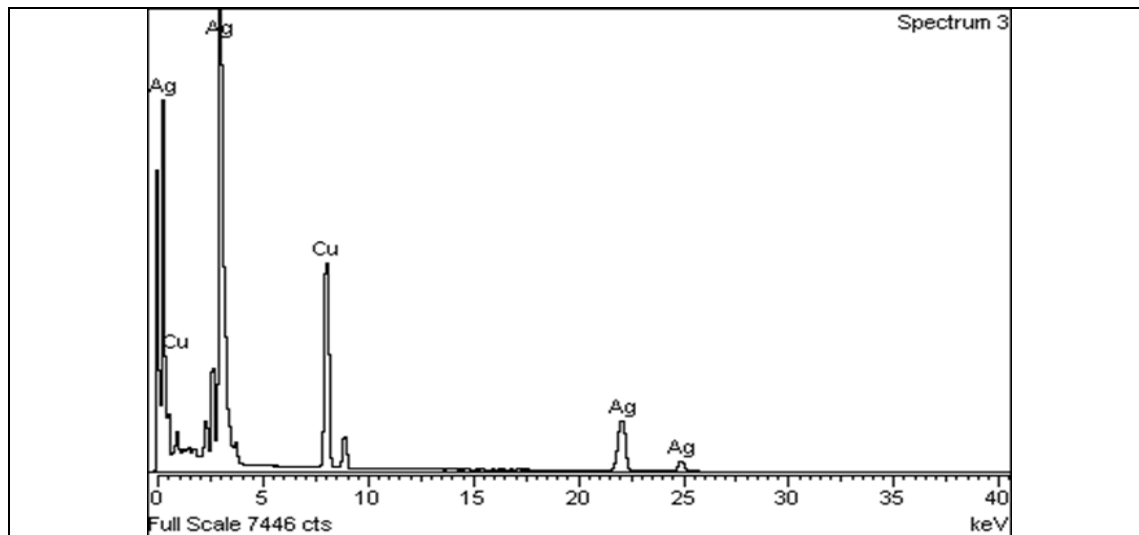
5.4.8.a X-ray spectrum from silver nanoparticles produced from leaf extract containing chromium and cadmium



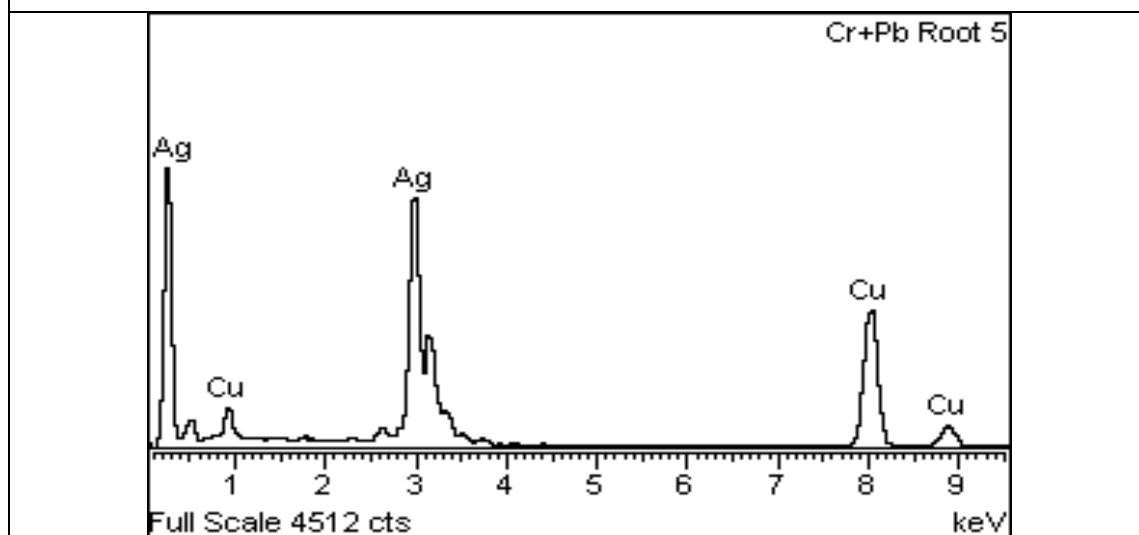
5.4.8.b X-ray spectrum from silver nanoparticles produced from root extract containing chromium and cadmium

The spectra do not show a peak for chromium at 5.44keV or cadmium peaks at 3.13 and 23.17keV, a silver peak at 2.98keV is present; the samples were mounted onto

copper microscope grids coated with an evaporated carbon film. The silver and cadmium K and L peaks overlap therefore the silver may mask the presence of cadmium.

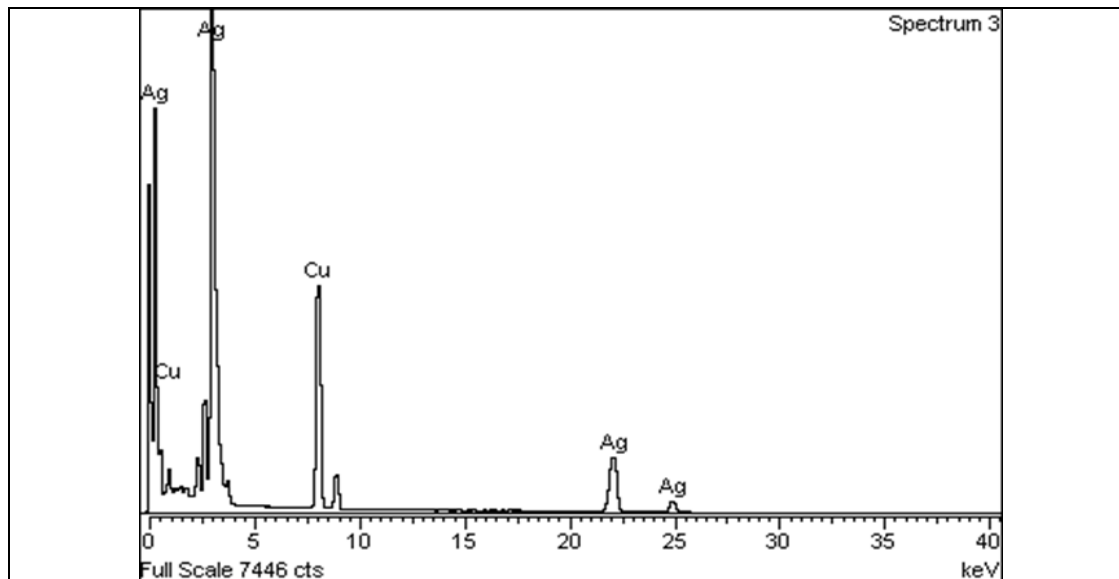


5.4.9.a X-ray spectrum from silver nanoparticles produced from leaf extract containing chromium and lead

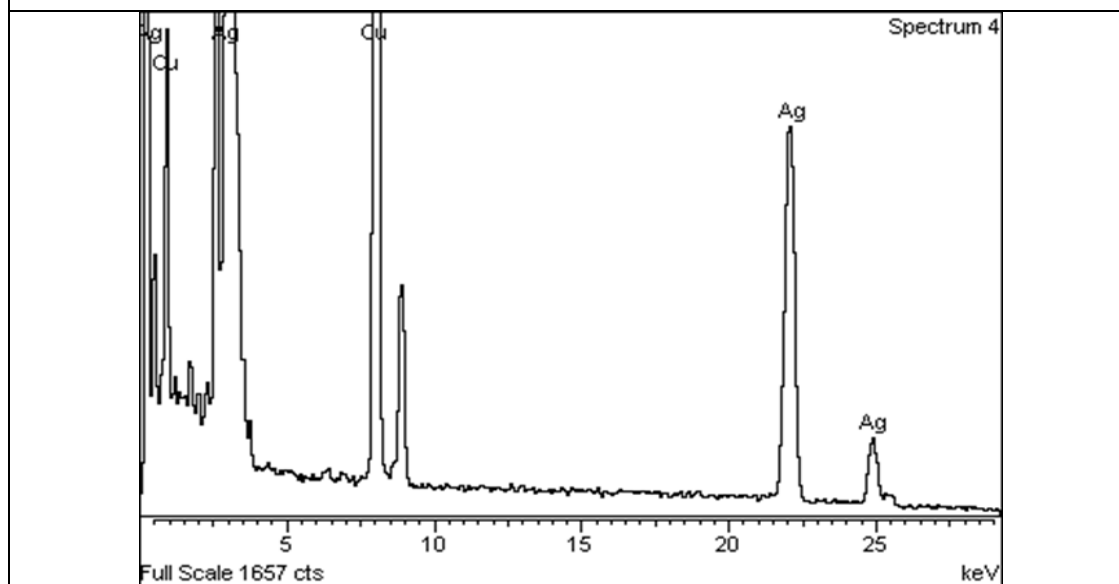


5.4.9.b X-ray spectrum from silver nanoparticles produced from root extract containing chromium and lead

The spectra do not show a chromium peak at 5.44keV or a lead peak at 2.34keV, a silver peak at 2.98keV is present; the samples were mounted onto copper microscope grids coated with an evaporated carbon film.

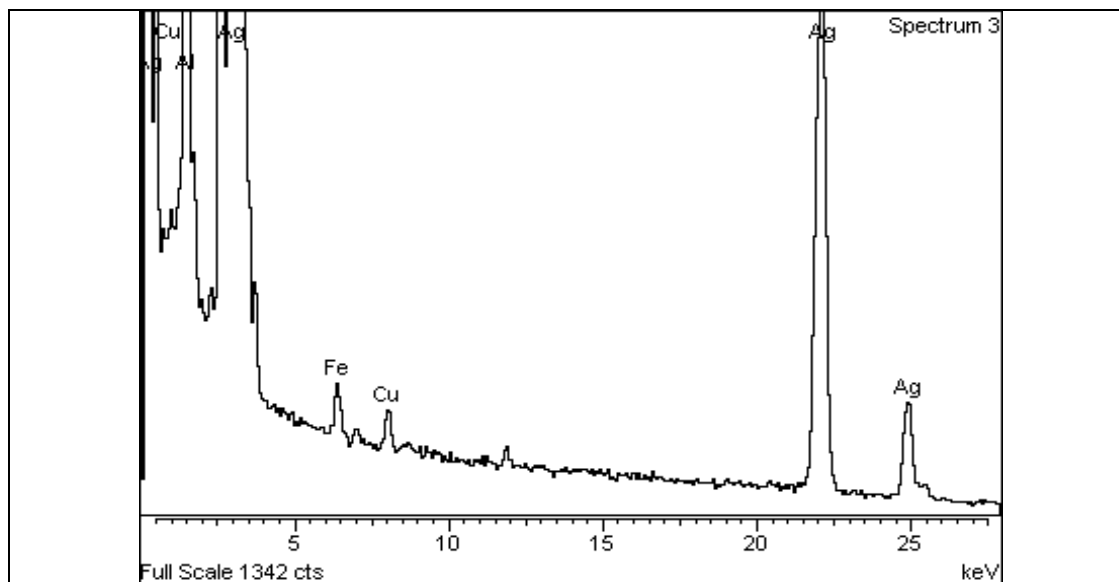


5.4.10.a X-ray spectrum from silver nanoparticles produced from leaf extract containing cadmium and lead

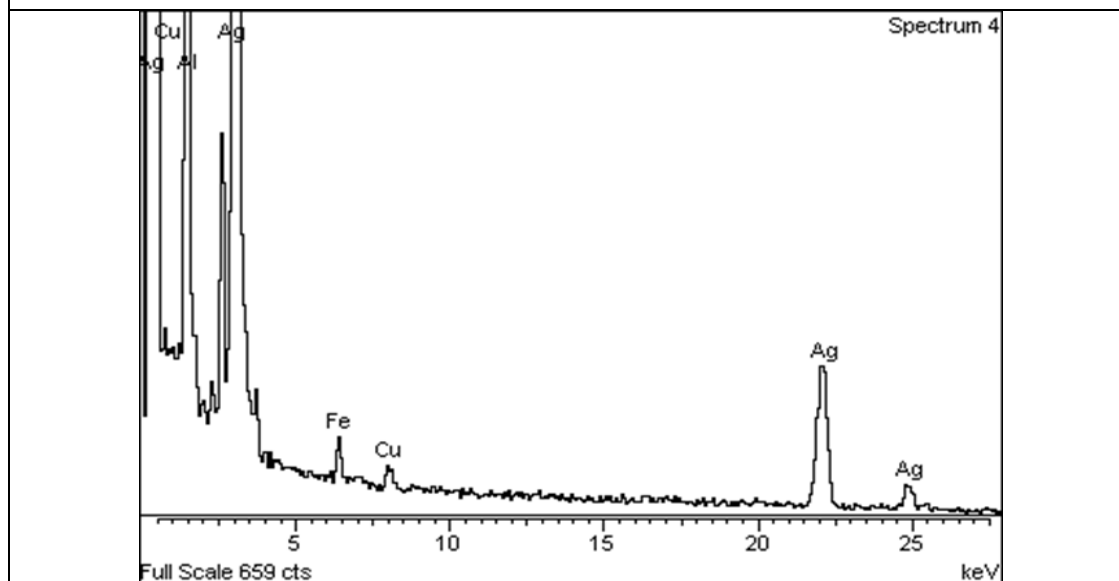


5.4.10.b X-ray spectrum from silver nanoparticles produced from root extract containing cadmium and lead

The spectra do not show a cadmium peak at 3.13keV or a lead peak at 10.55keV, a silver peak at 2.98keV is present; the samples were mounted onto copper microscope grids coated with an evaporated carbon film. The silver and cadmium K and L peaks overlap therefore the silver may mask the presence of cadmium.

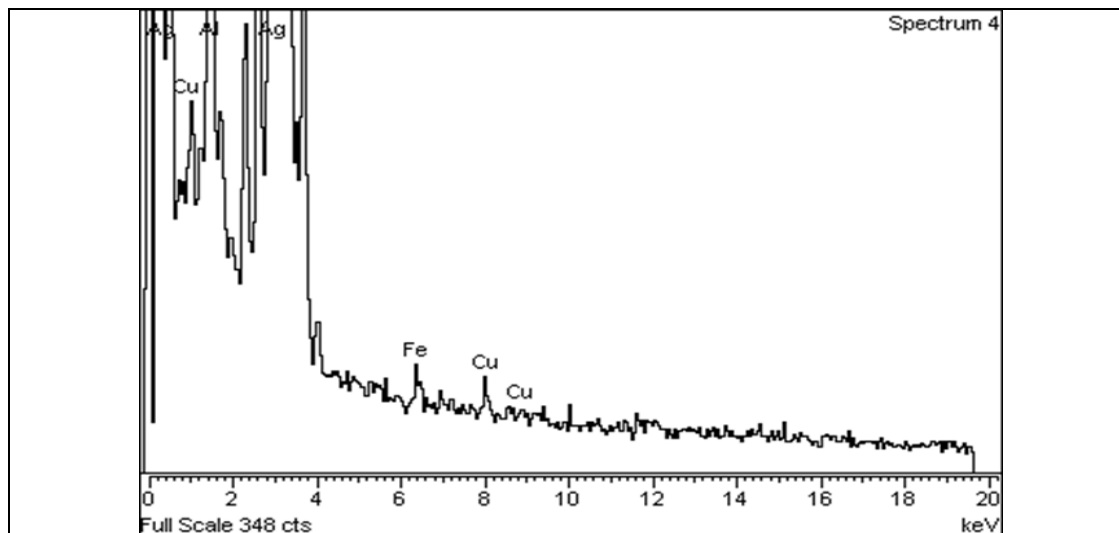


5.4.11.a X-ray spectrum from silver nanoparticles produced from leaf extract containing copper, chromium and cadmium

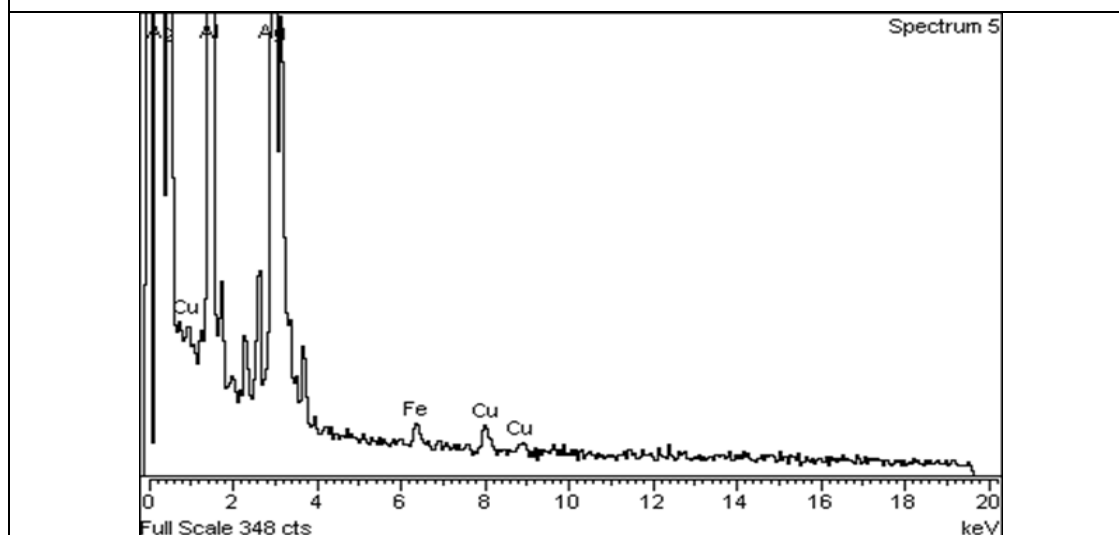


5.4.11.b X-ray spectrum from silver nanoparticles produced from root extract containing copper, chromium and cadmium

The spectra do not show a chromium peak at 5.44keV or cadmium peaks at 3.13 or 23.17keV. A copper peak at 8.04keV is present, a silver peak at 2.98keV is present, and the samples were mounted onto aluminium microscope grids coated with an evaporated carbon film. The silver and cadmium K and L peaks overlap therefore the silver may mask the presence of cadmium.

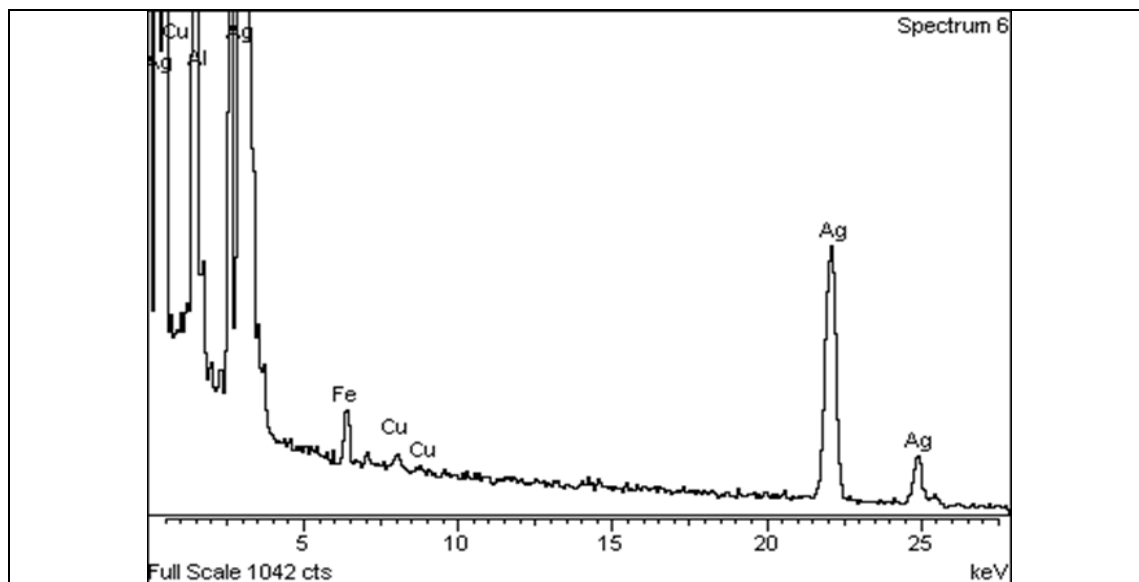


5.4.12.a X-ray spectrum from silver nanoparticles produced from leaf extract containing copper, chromium and lead

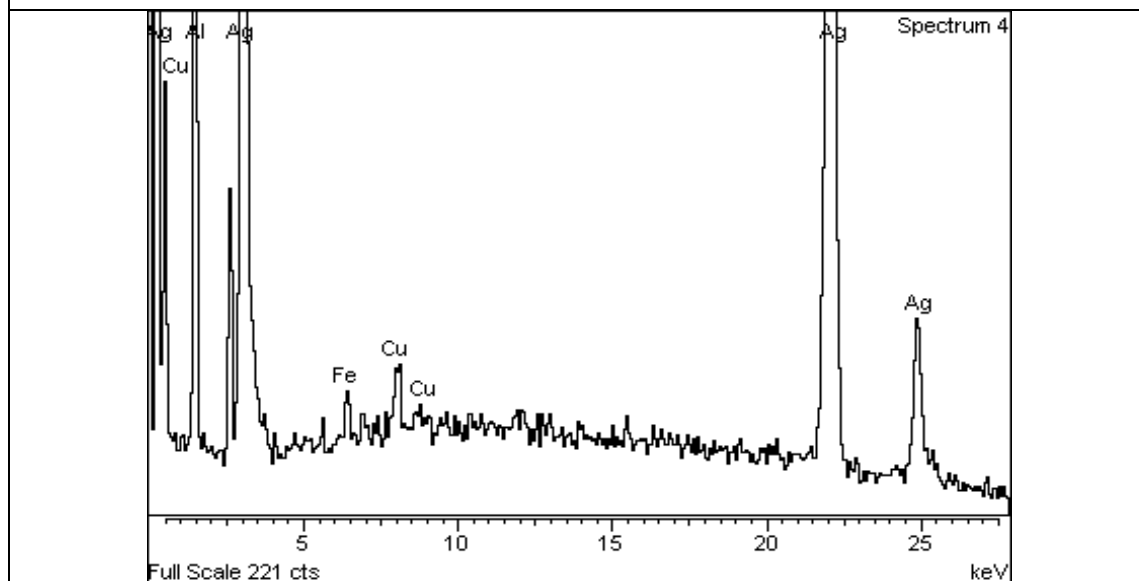


5.4.12.b X-ray spectrum from silver nanoparticles produced from root extract containing copper, chromium and lead

The spectra do not show a chromium peak at 5.44keV or a lead at 10.55keV. A copper peak at 8.04keV is present, a silver peak at 2.98keV is present, and the samples were mounted onto aluminium microscope grids coated with an evaporated carbon film.

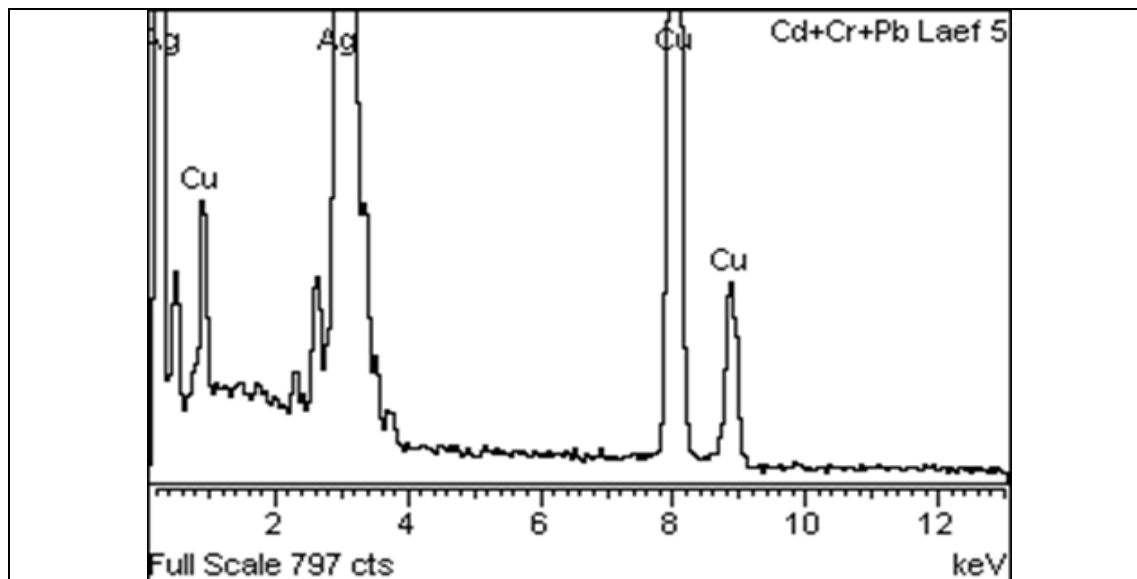


5.4.13.a X-ray spectrum from silver nanoparticles produced from leaf extract containing copper, cadmium and lead

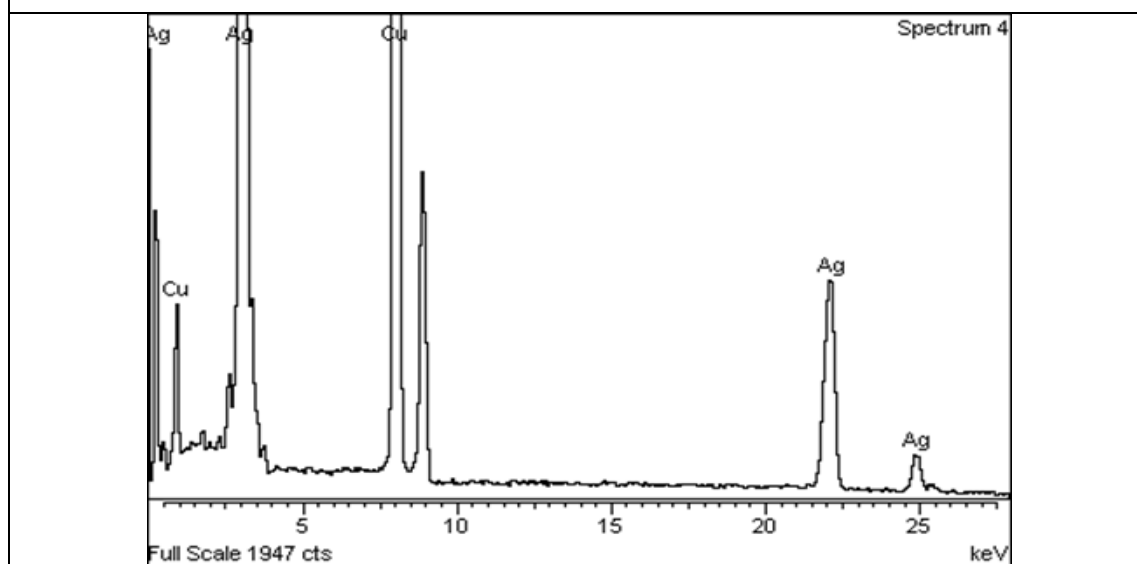


5.4.13.b X-ray spectrum from silver nanoparticles produced from root extract containing copper, cadmium and lead

The spectra do not show a cadmium peak at 3.13keV or a lead peak at 10.55keV. A copper peak at 8.04keV is present, a silver peak at 2.98keV is present, and the samples were mounted onto aluminium microscope grids coated with an evaporated carbon film. The silver and cadmium K and L peaks overlap therefore the silver may mask the presence of cadmium.



5.4.14.a X-ray spectrum from silver nanoparticles produced from leaf extract containing chromium, cadmium and lead



5.4.14.b X-ray spectrum from silver nanoparticles produced from root extract containing chromium, cadmium and lead

The spectra do not show a cadmium peak at 3.13keV or chromium peak at 5.44keV or a lead peak at 10.55keV. A silver peak at 2.98keV is present; the samples were mounted onto copper microscope grids coated with an evaporated carbon film. The silver and cadmium K and L peaks overlap therefore the silver may mask the presence of cadmium.

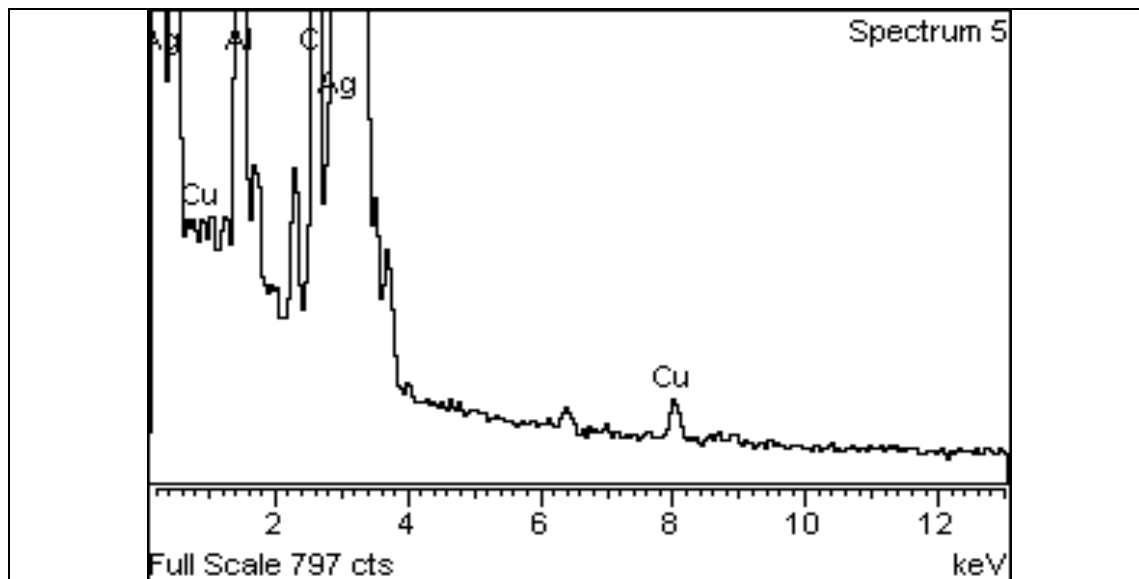


Fig 5.4.15.a X-ray spectrum from silver nanoparticles produced from leaf extract containing all metals

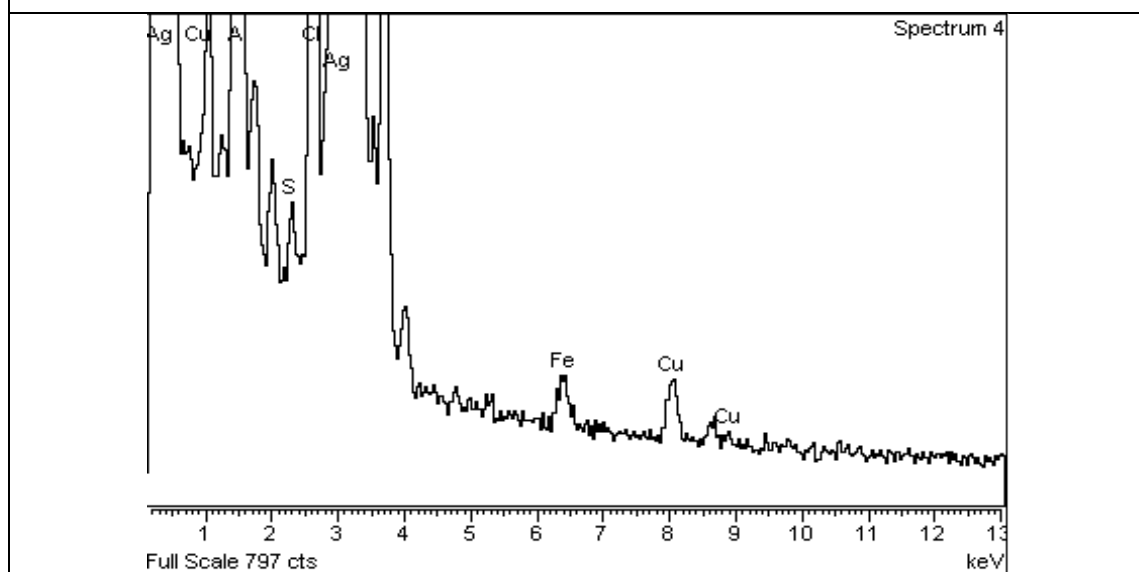


Fig 5.4.15.b X-ray spectrum from silver nanoparticles produced from root extract containing all metals

The spectra do not show a cadmium peak at 3.13keV a chromium peak at 5.44keV or a lead peak at 10.55keV. A copper peak is shown at 8.04keV. A silver peak at 2.98keV is present; the samples were mounted onto aluminium microscope grids coated with an evaporated carbon film. The silver and cadmium K and L peaks overlap therefore the silver may mask the presence of cadmium.

5.5 TEM results for metal location in plants

Following results are obtained for selected combinations to check the location of accumulated metals in PA plants. Leaf and root sections are checked in TEM for determining the location. The below figures and charts are examples of the results obtained from the leaf and root sections. Analysis of all of the leaf sections cut from PA leaves that had been hydroponically grown, except the lead sample (Fig 5.5.2 and Chart 5.5.2) show no evidence of metal accumulation and are not shown in the results section.

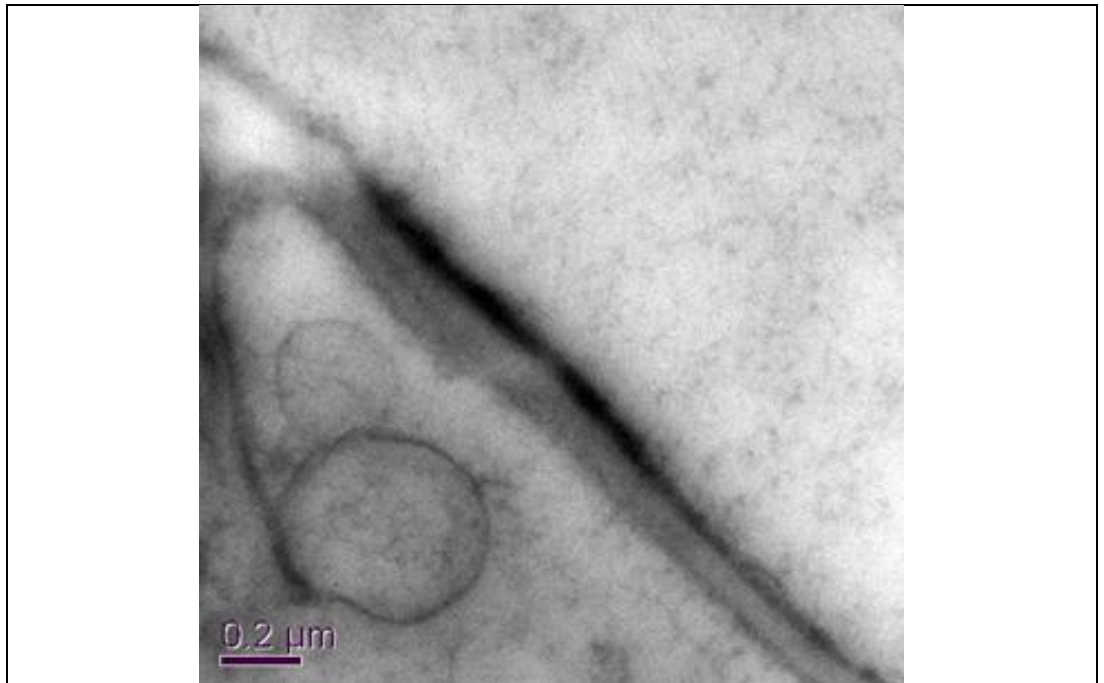


Fig 5.5.1

Cr leaf semi-thin section

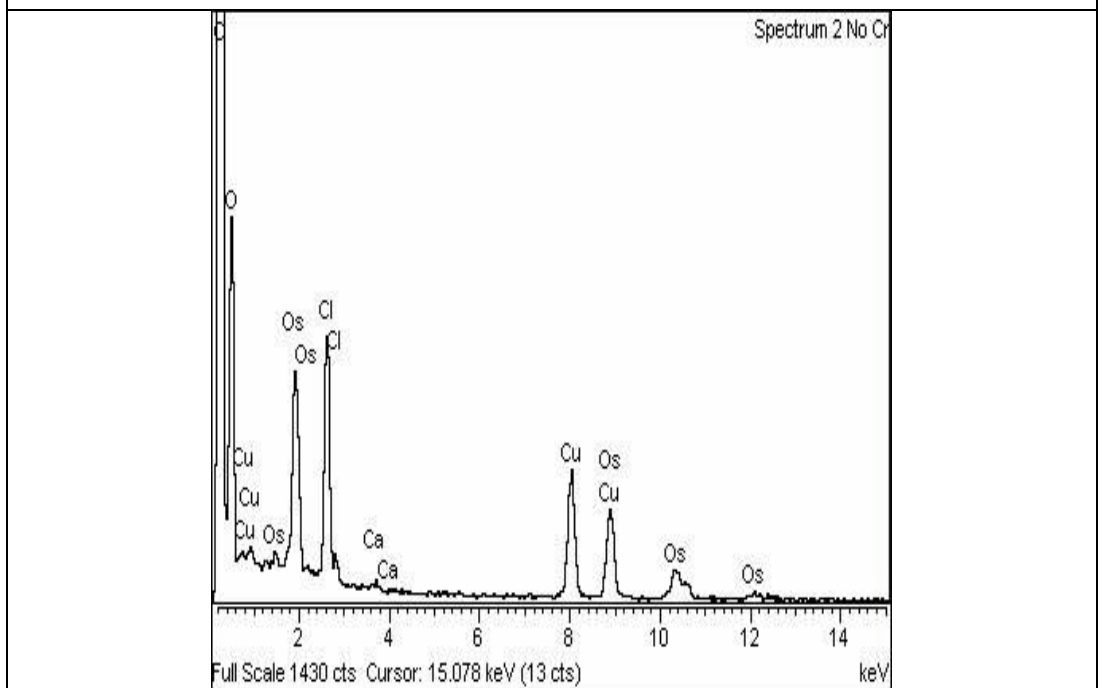


Chart 5.5.1

X-ray spectrum from Cr Leaf section

Fig 5.5.1 is a micrograph of a semithin section of PA leaf after hydroponic culture in Cr solution. The X-ray spectrum acquired from the cell membrane arrowed (Chart 5.5.1) does not show a peak for Cr at 5.41 keV.

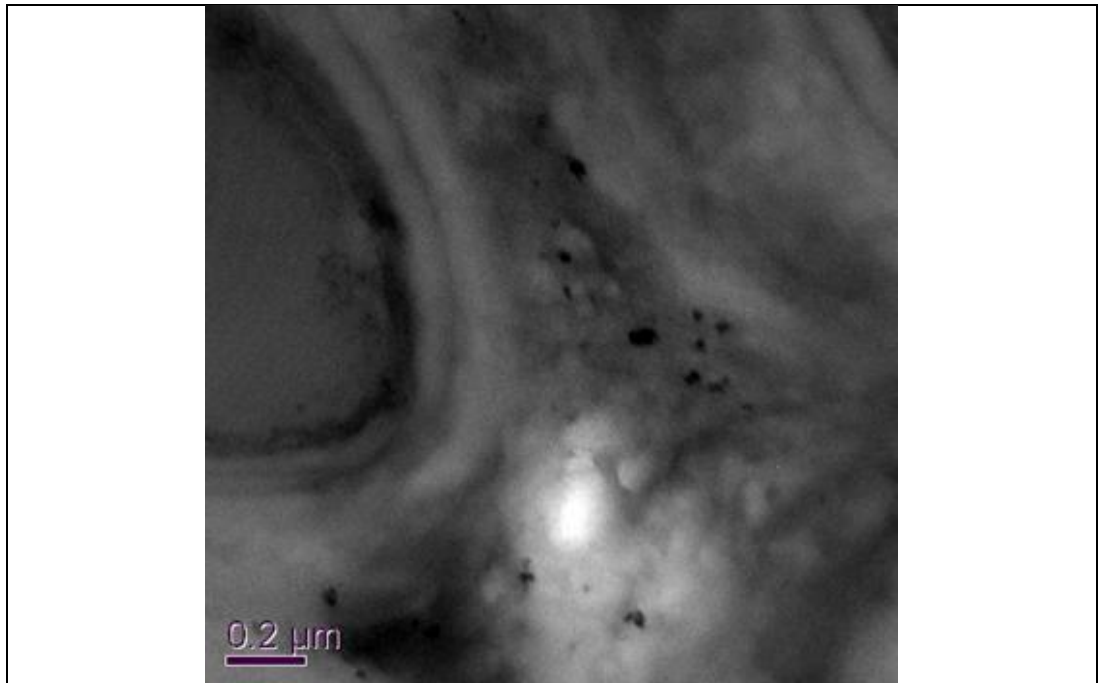


Fig 5.5.2 Pb leaf semi-thin section

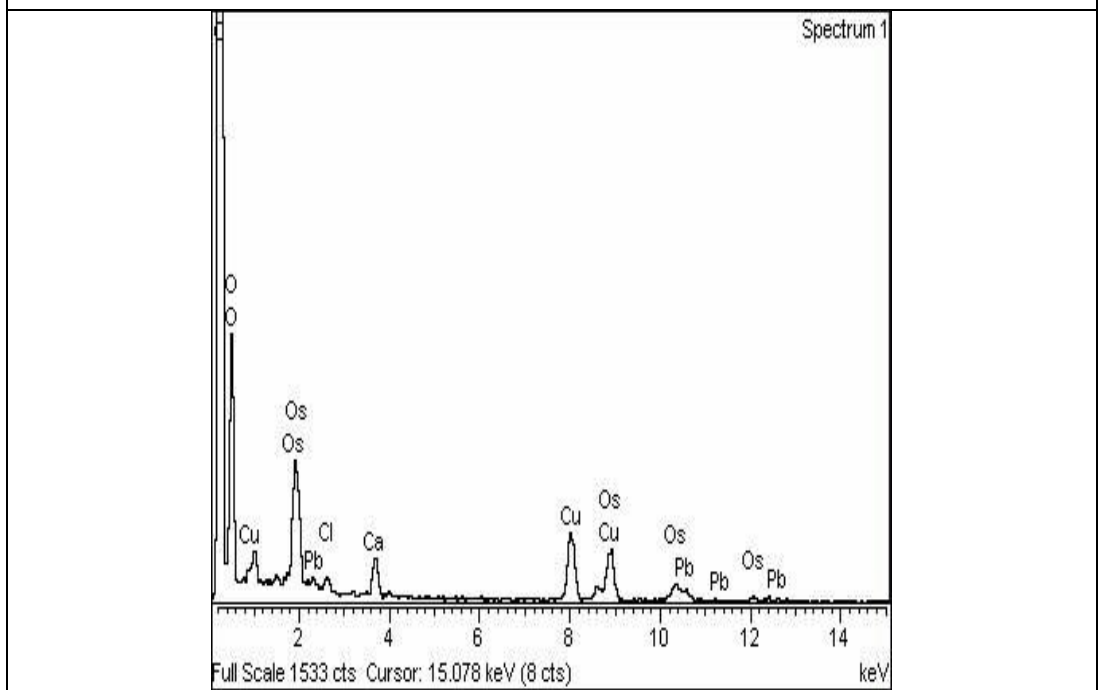


Chart 5.5.2 X-ray spectrum from Pb Leaf section

Fig 5.5.2 is a micrograph of a section cut from the leaf of a PA plant grown hydroponically in lead solution. Pb particles are visible in the cell wall (Chart 5.5.2). The lead and osmium X-ray peaks shown have overlapping peaks between 8 and 13 keV due to the complex L series having numerous characteristic peaks.

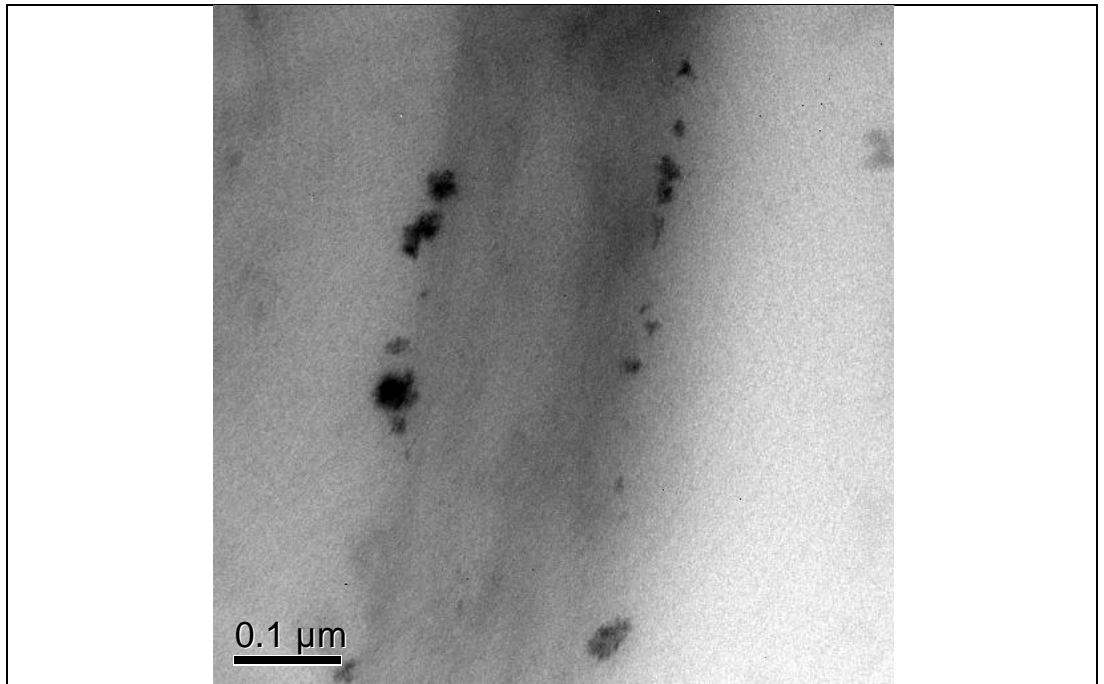


Fig 5.5.3 **Cu Root semi-thin section**

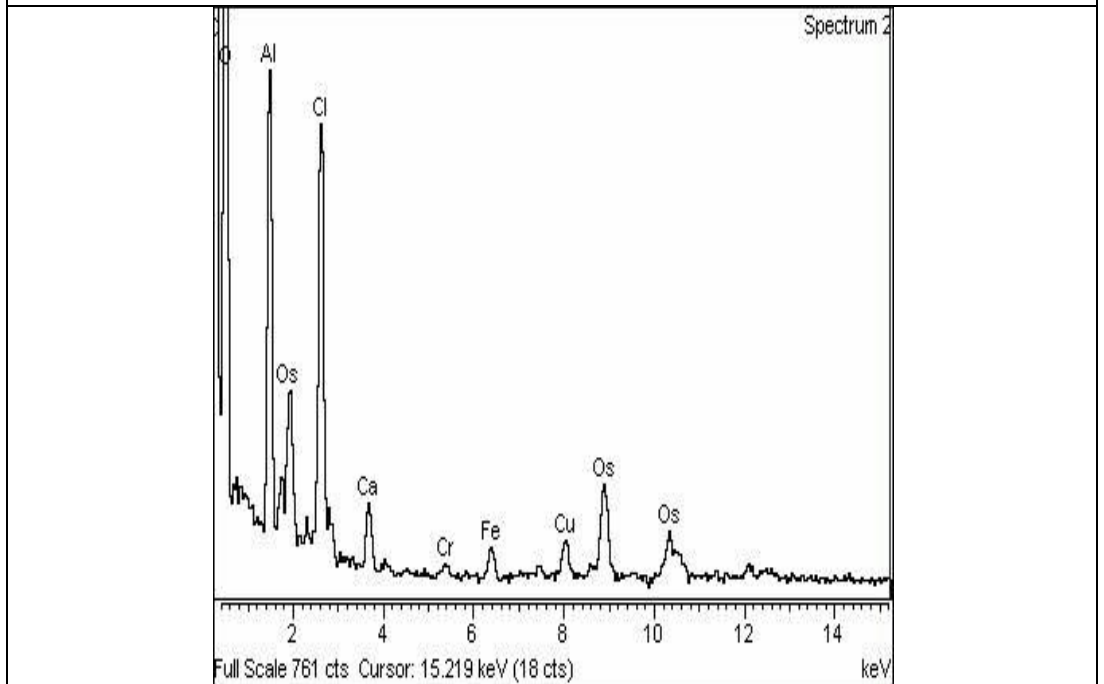


Chart 5.5.3 **X-ray spectrum from Cu Root section**

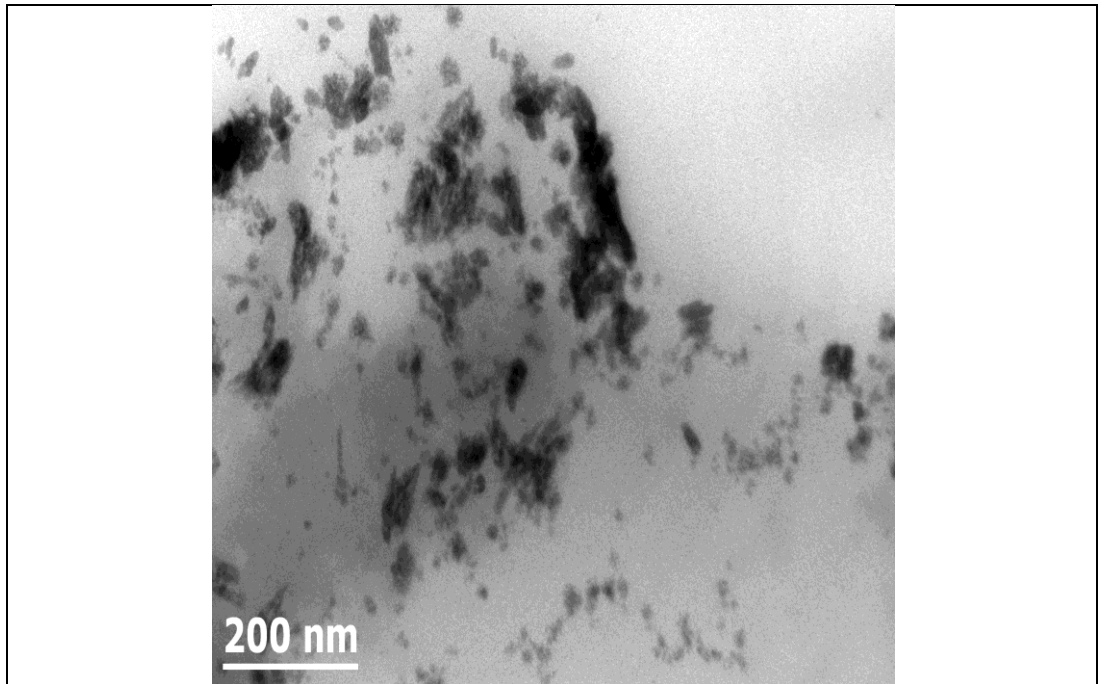


Fig 5.5.4 **Cr Root semi-thin section**

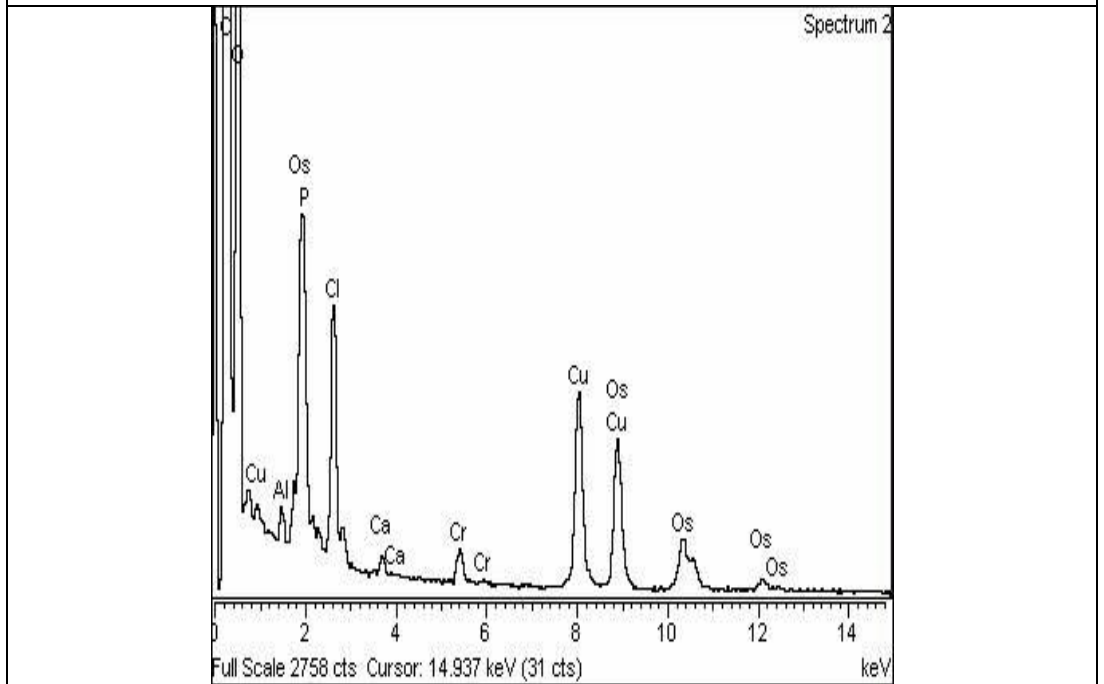


Chart 5.5.4 **X-ray spectrum from Cr Root section**

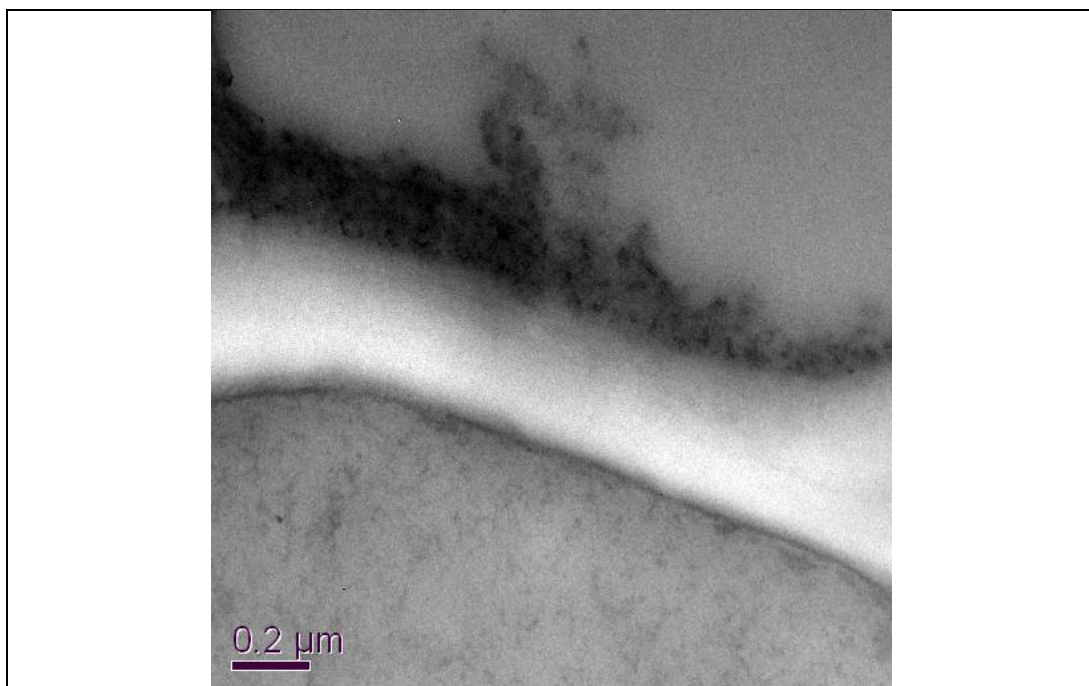


Fig 5.5.5

Cd Root semi-thin section

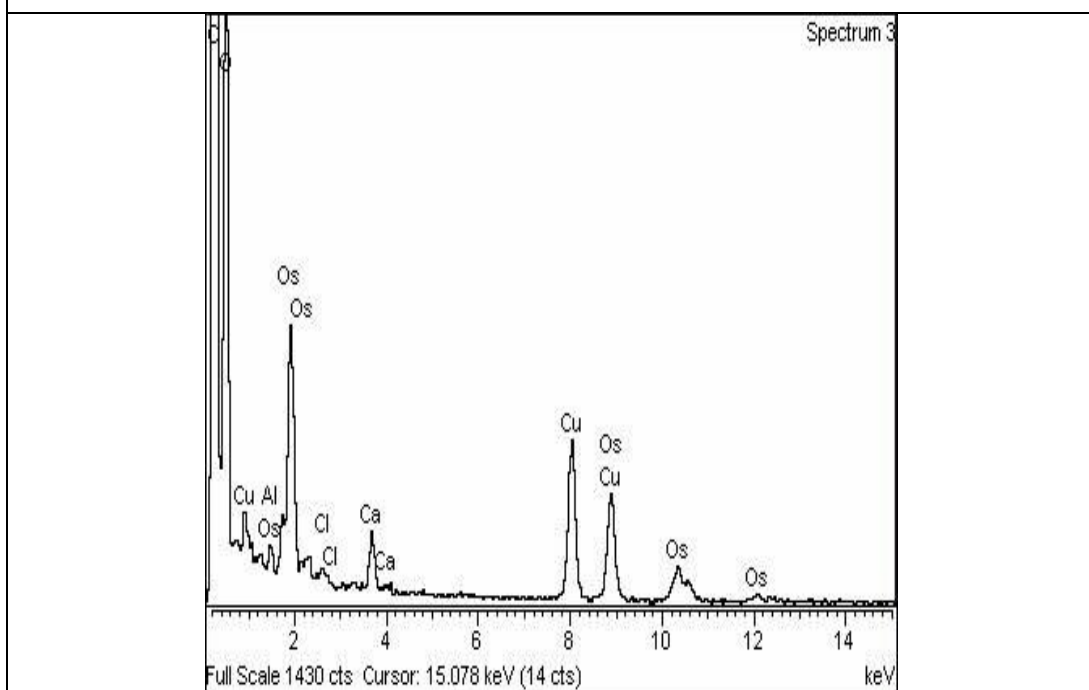


Chart 5.5.5

X-ray spectrum from Cd Root section

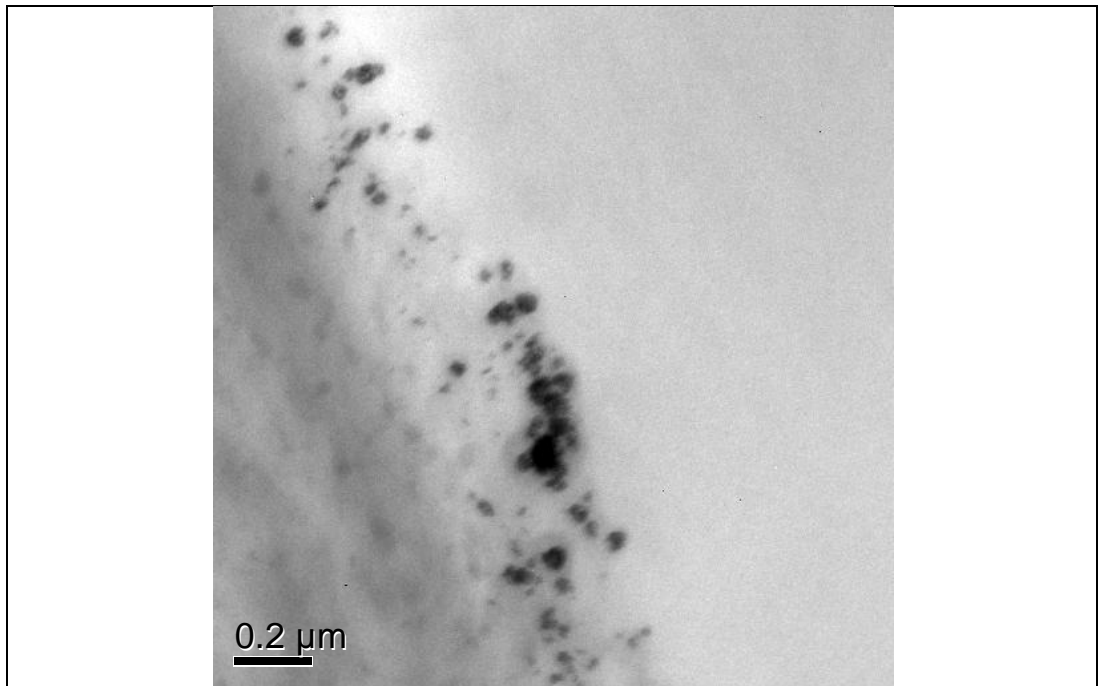


Fig 5.5.6 Pb Root semi-thin section

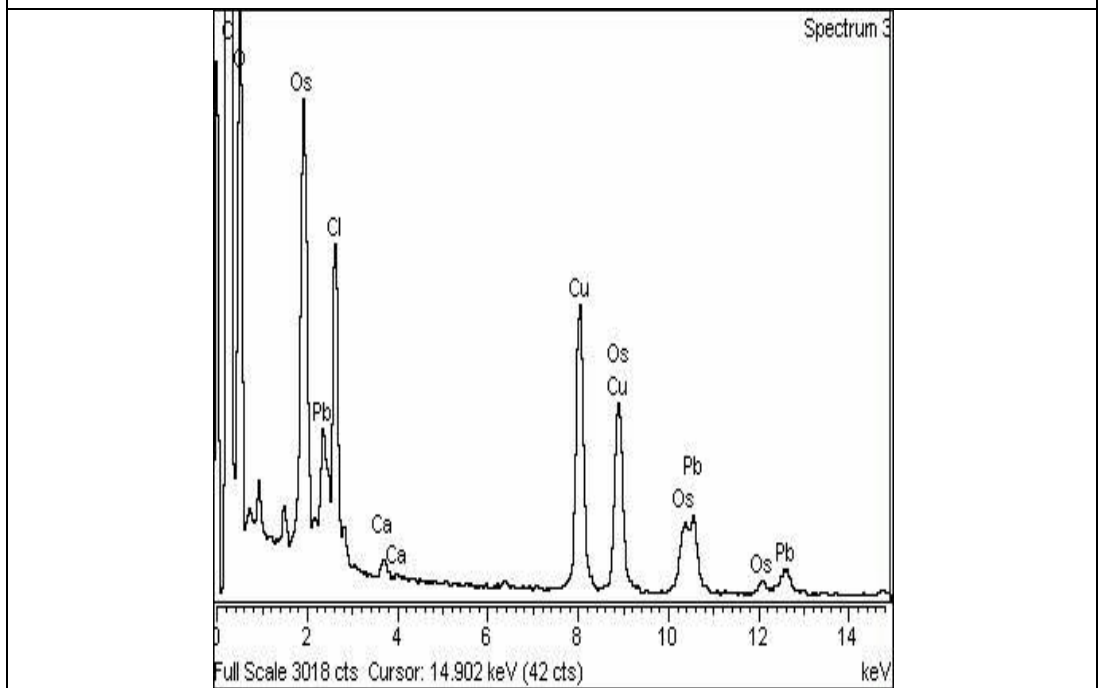


Chart 5.5.6 X-ray spectrum from Pb Root section

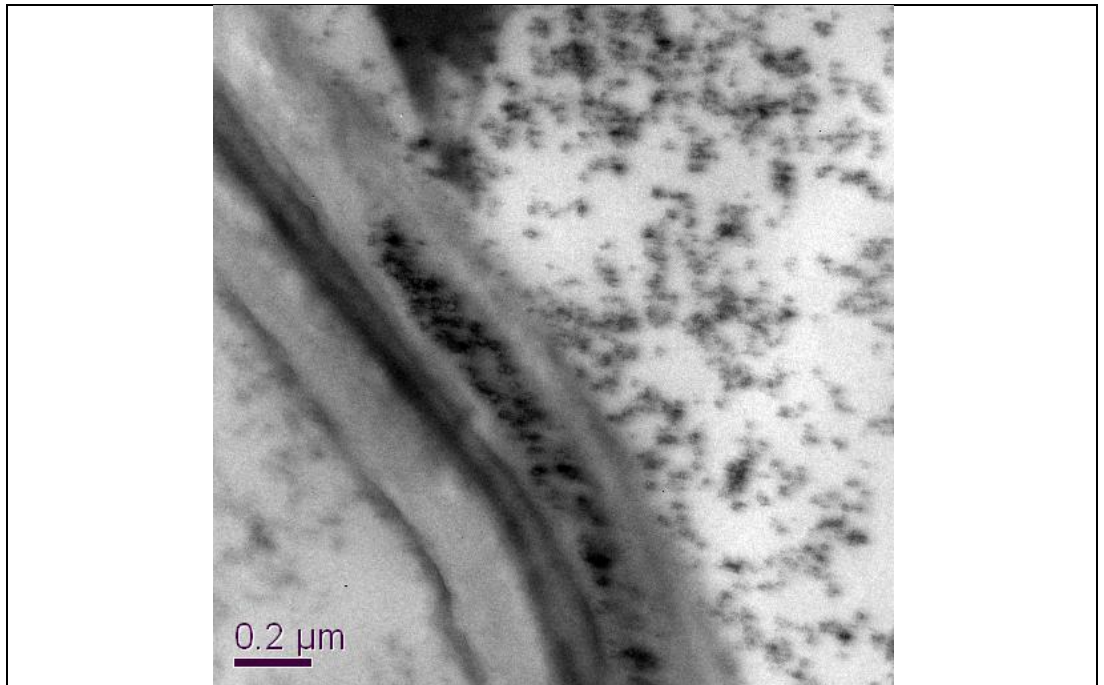


Fig 5.5.7 **Cu+Cr Root semi-thin section**

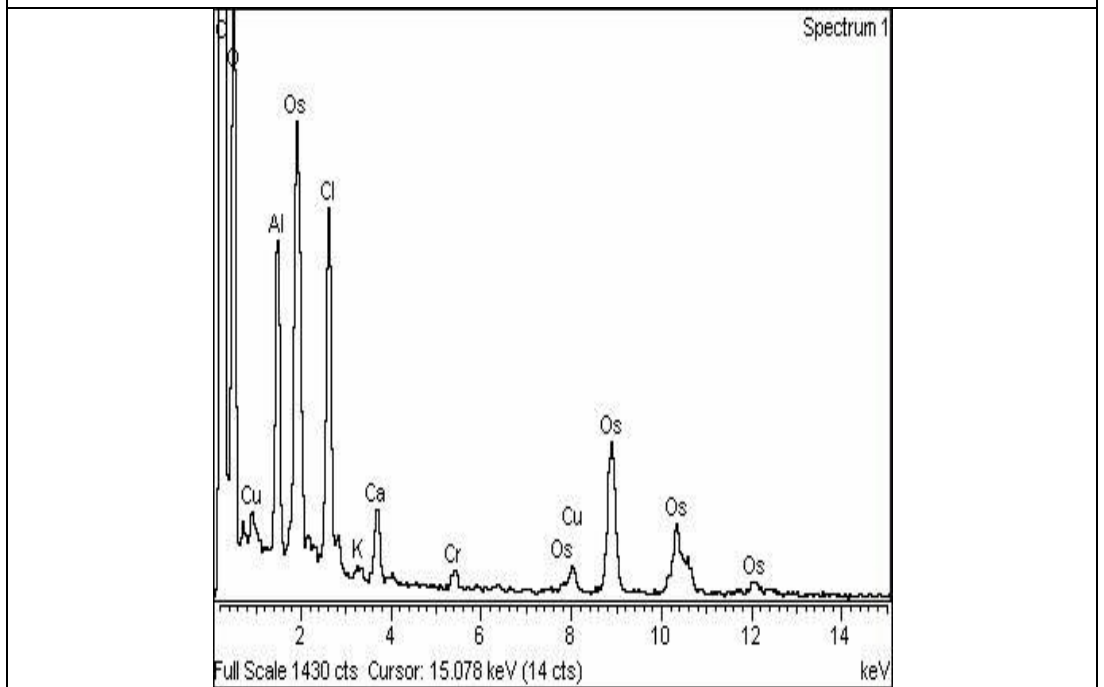


Chart 5.5.7 **X-ray spectrum from Cu+Cr Root section**

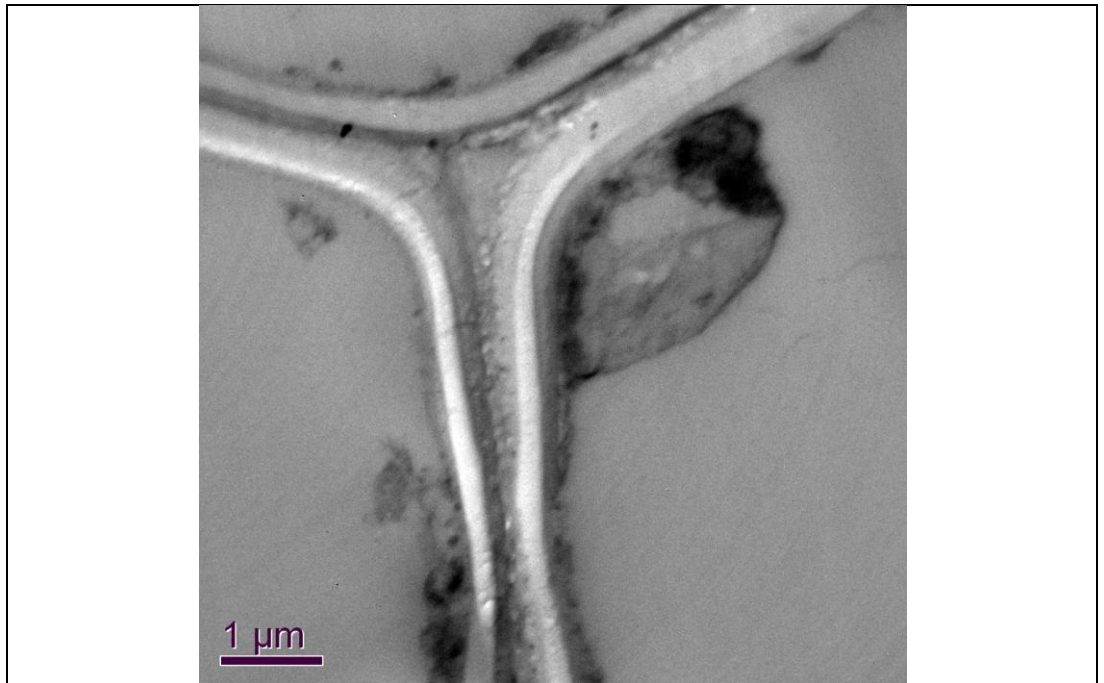


Fig 5.5.8 **Cu+Cd Root semi-thin section**

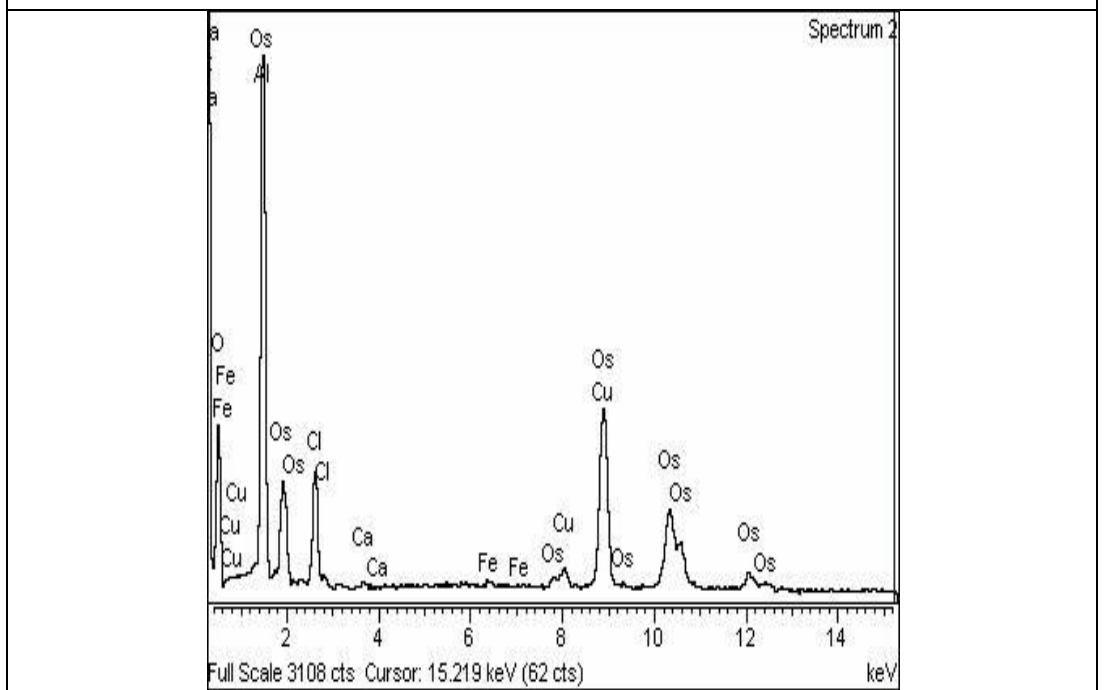


Chart 5.5.8 **X-ray spectrum from Cu+Cd Root section**

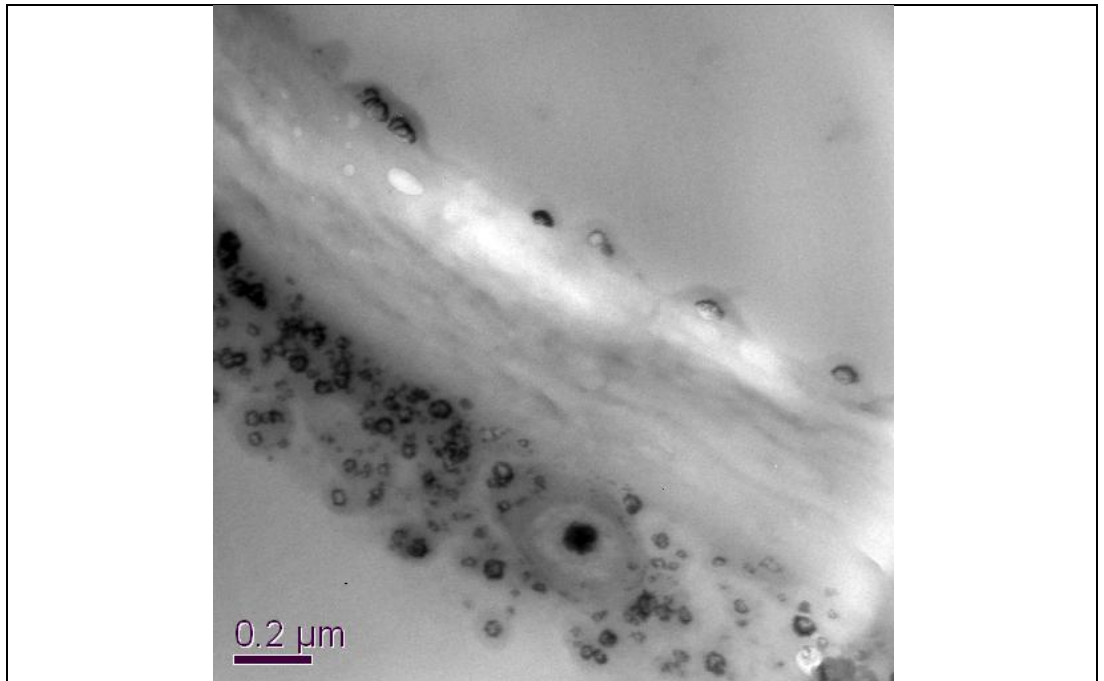


Fig 5.5.9 **Cu+Pb Root semi-thin section**

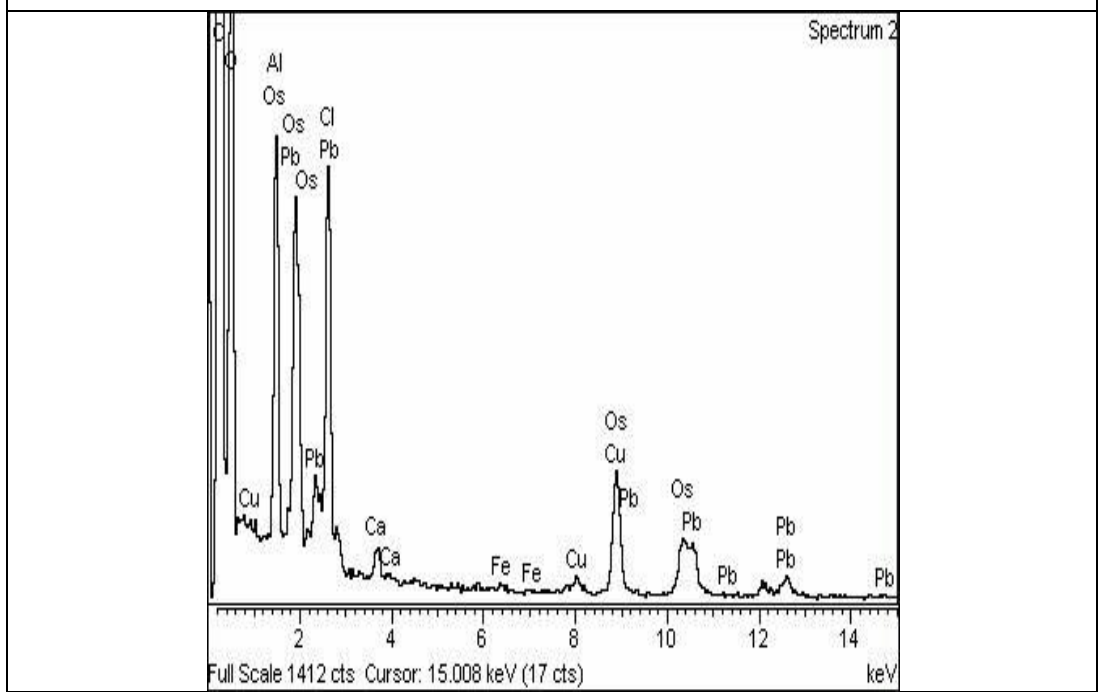


Chart 5.5.9 **X-ray spectrum from Cu+Pb Root section**

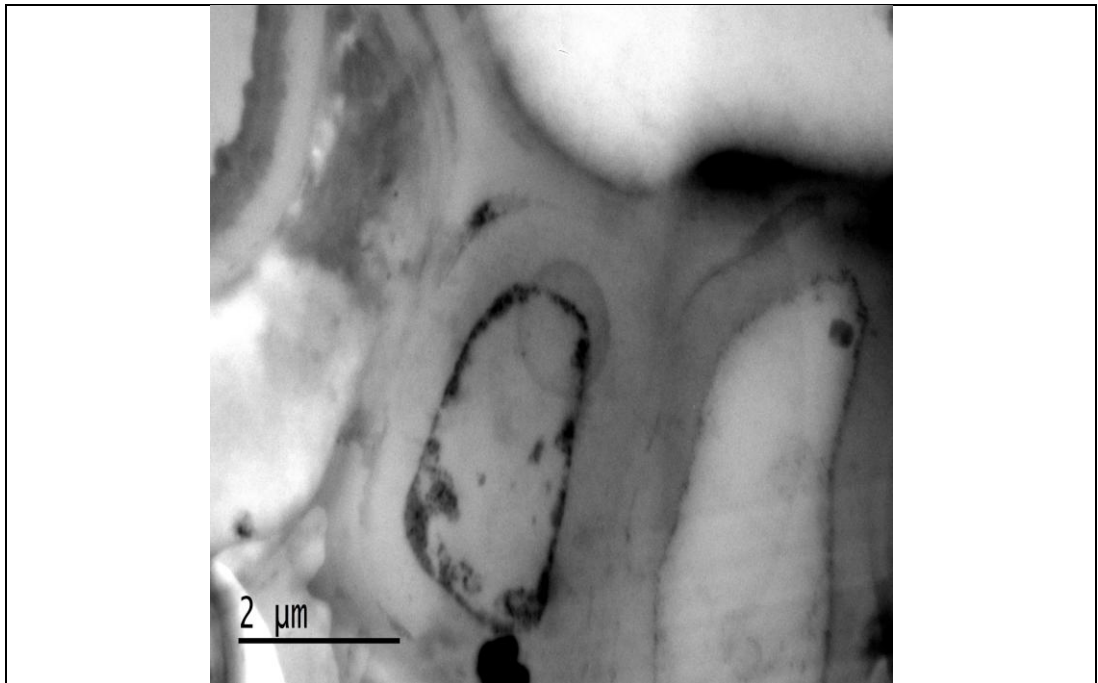


Fig 5.5.10 Cd+Cr Root semi-thin section

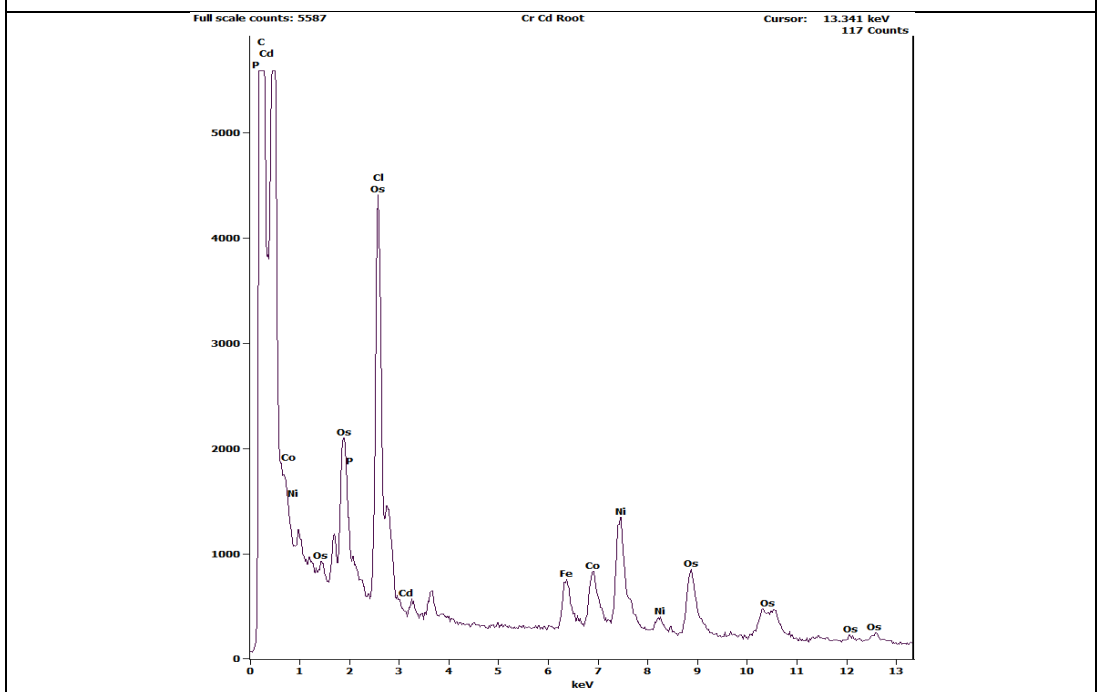


Chart 5.5.10 X-ray spectrum from Cd+Cr Root section

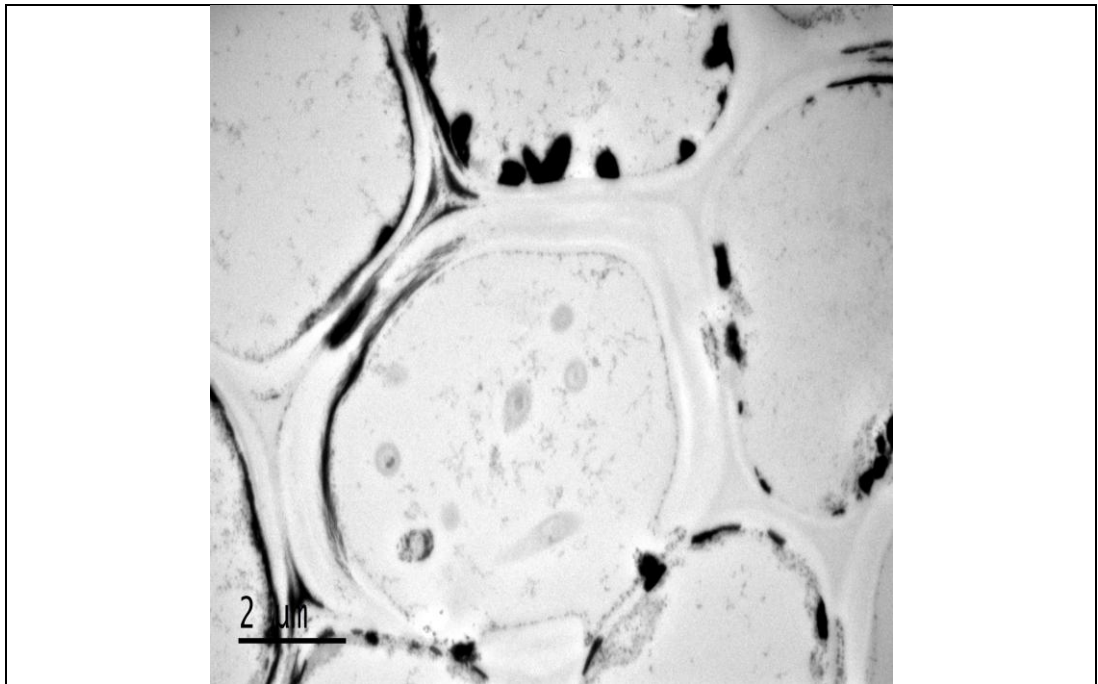


Fig 5.5.11 Cr+Pb Root semi-thin section

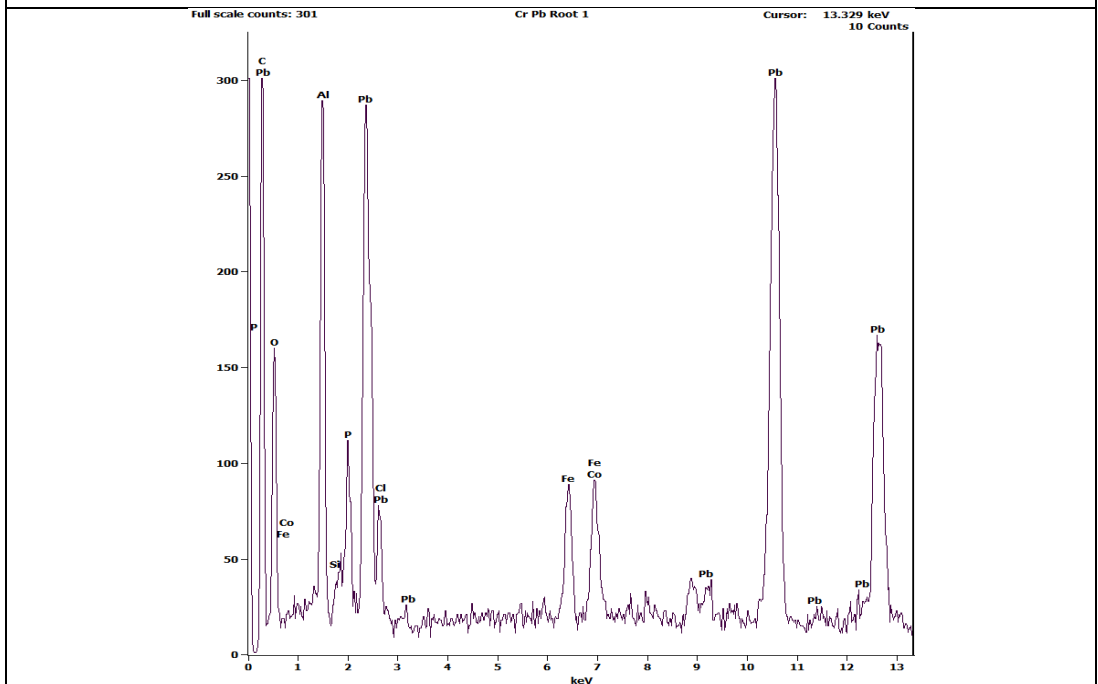


Chart 5.5.11 X-ray spectrum from Cr+Pb Root section

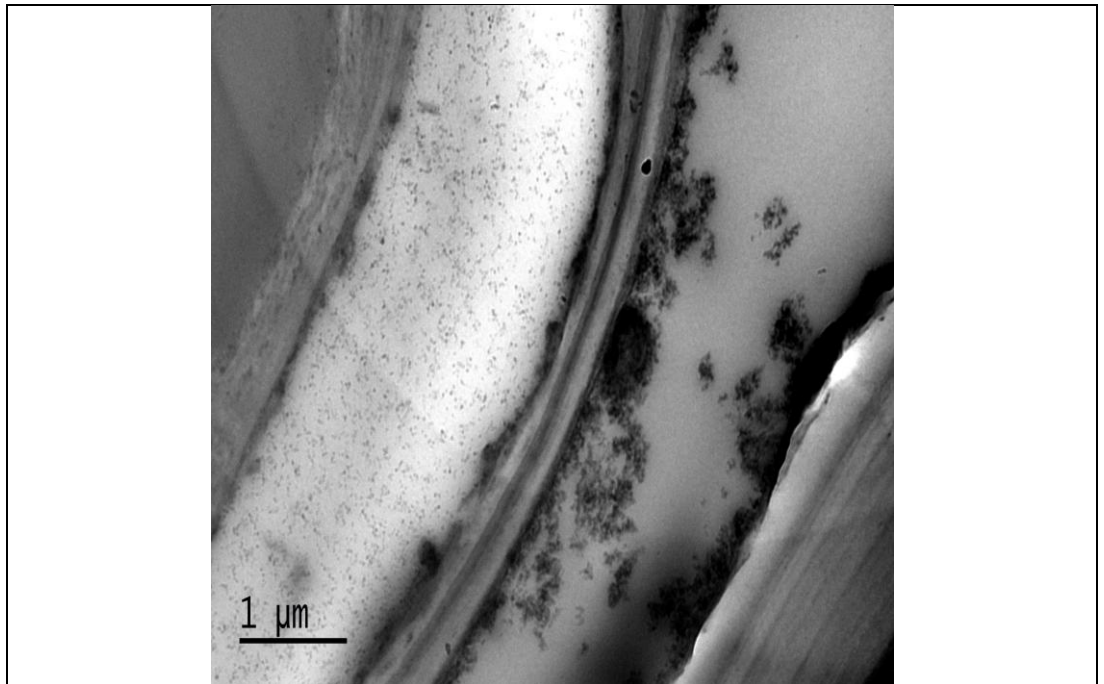


Fig 5.5.12 Cd+Pb Root semi-thin section

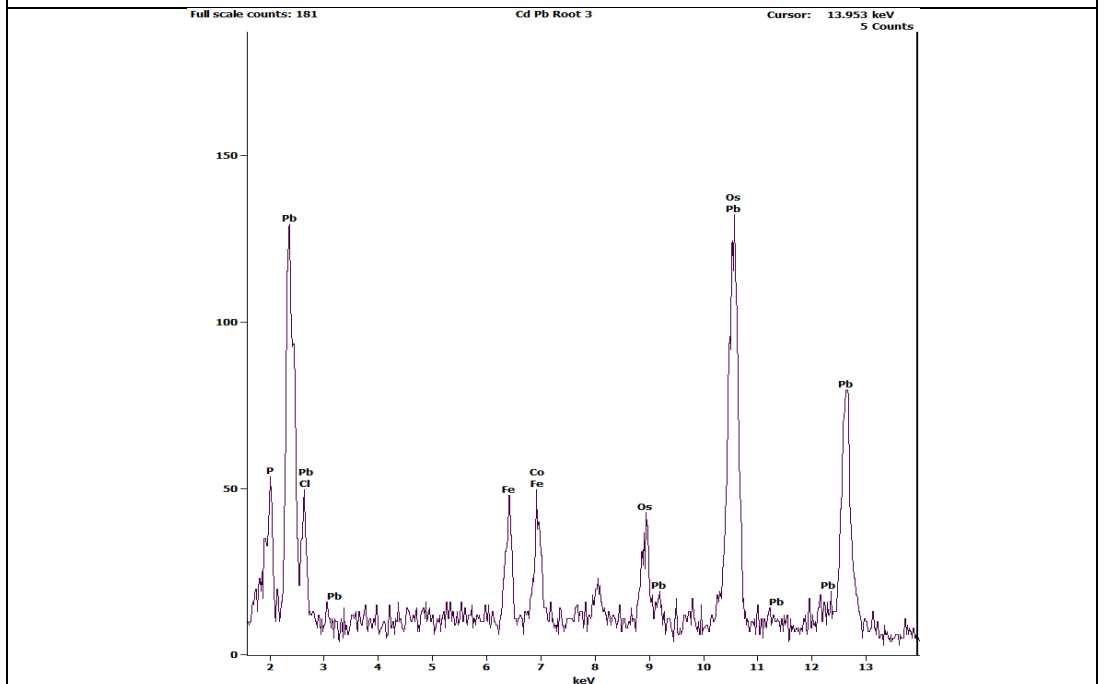


Chart 5.5.12 X-ray spectrum from Cd+Pb Root section

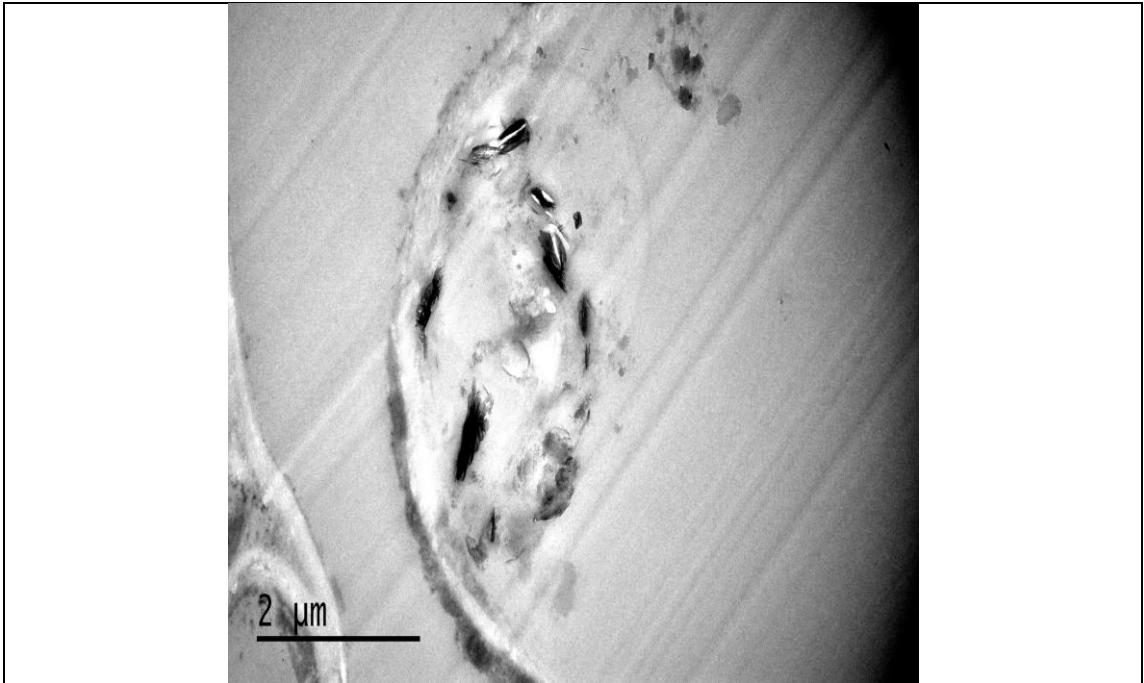


Fig 5.5.13 Cd+Cr+Cu Root semi-thin section

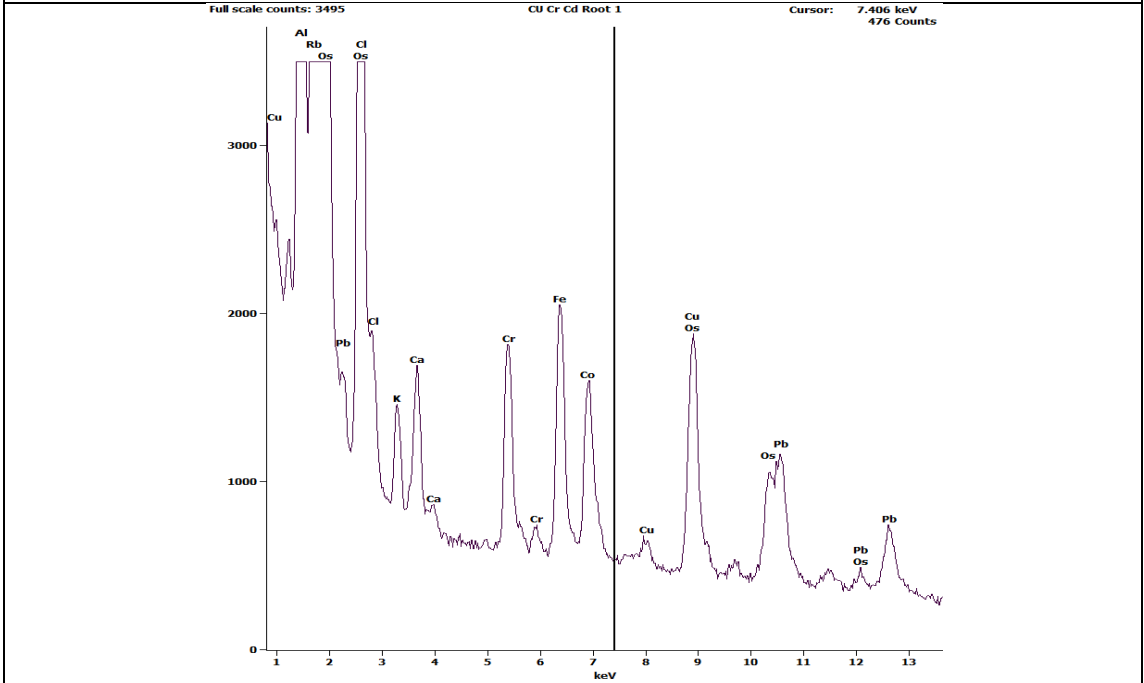


Chart 5.5.13 X-ray spectrum from Cd+Cr+Cu Root section

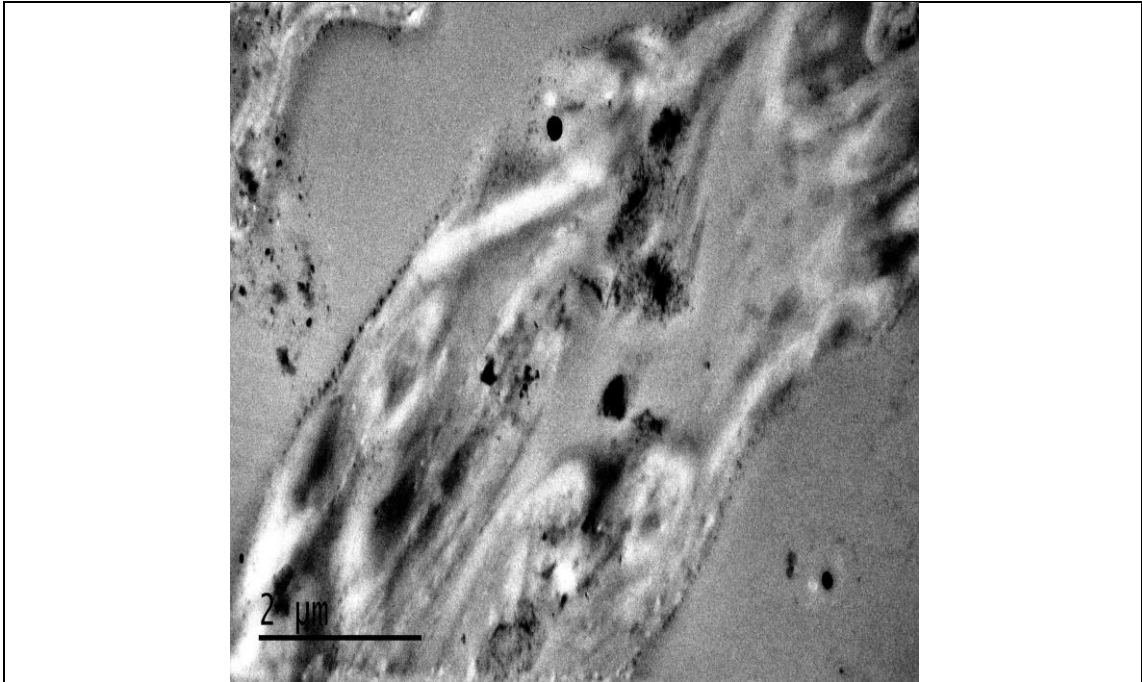


Fig 5.5.14 Cr+Cu+Pb Root semi-thin section

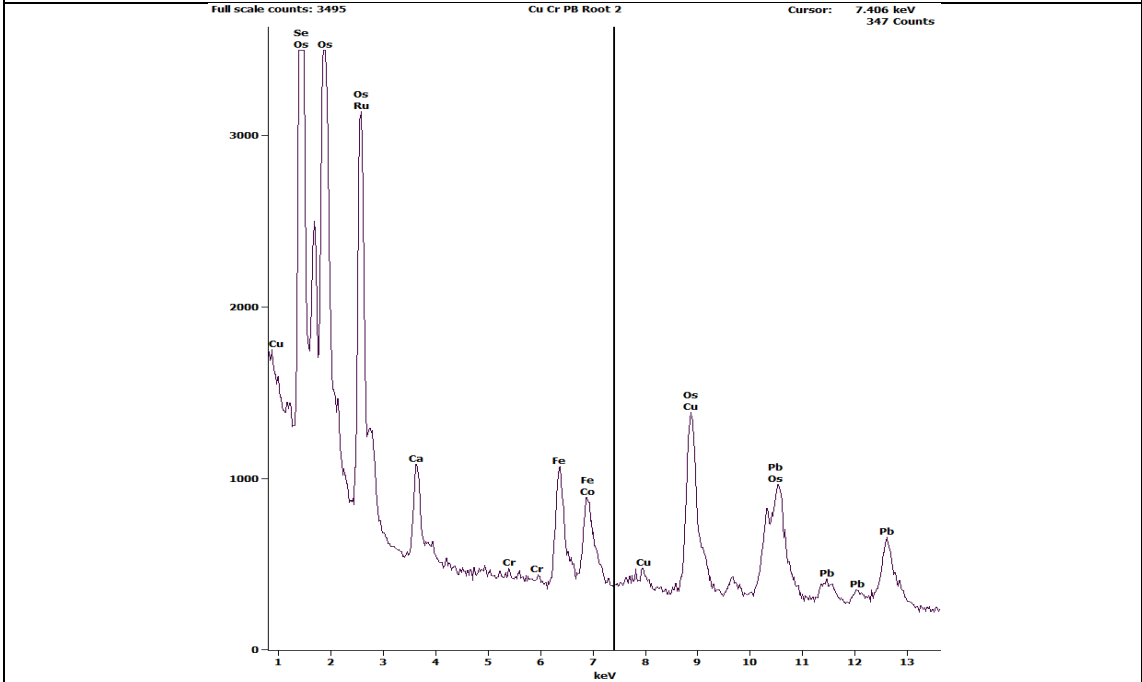


Chart 5.5.14 X-ray spectrum from Cr+Cu+Pb Root section

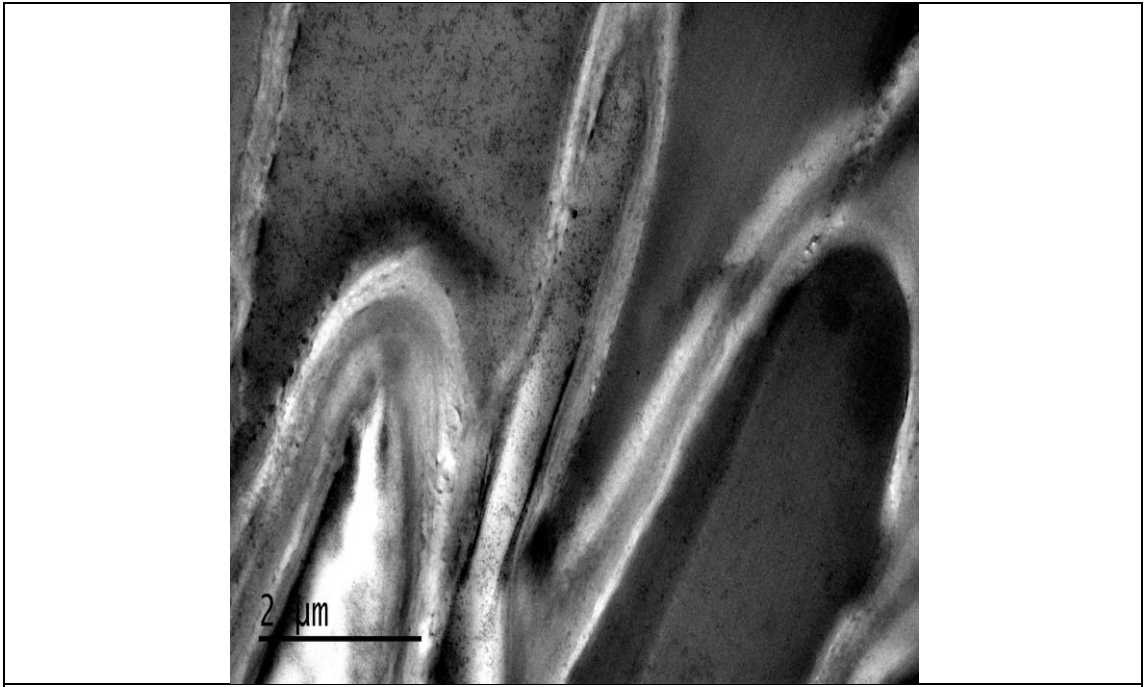


Fig 5.5.15 Cd+Cu+Pb Root semi-thin section

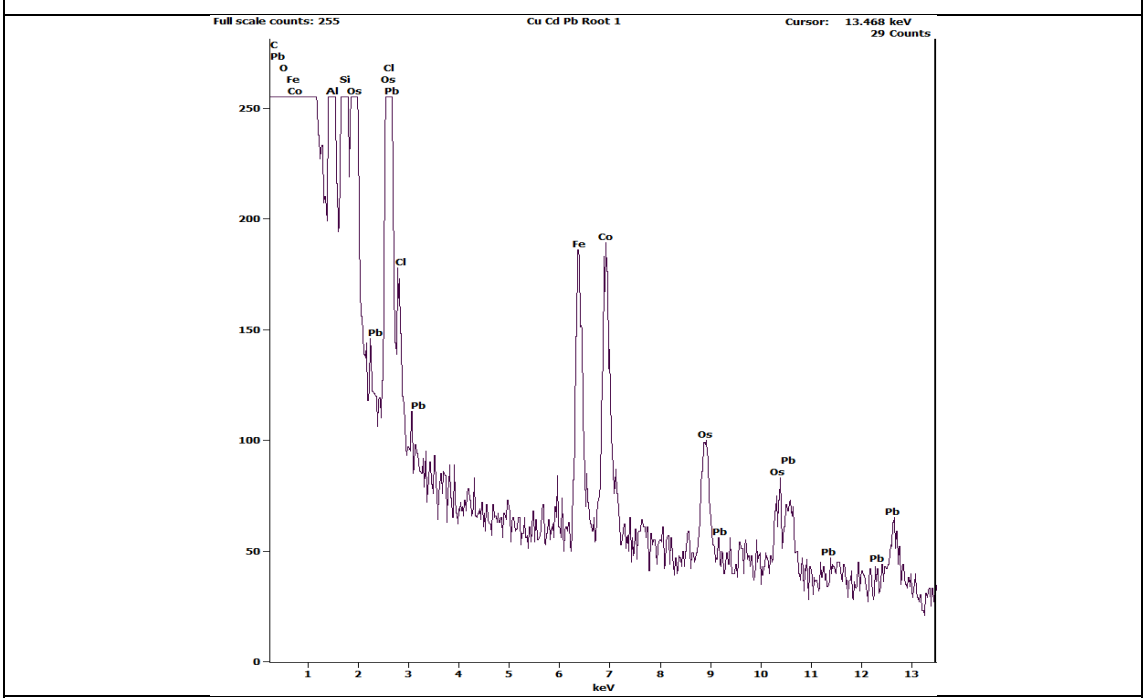


Chart 5.5.15 X-ray spectrum from Cd+Cu+Pb Root section

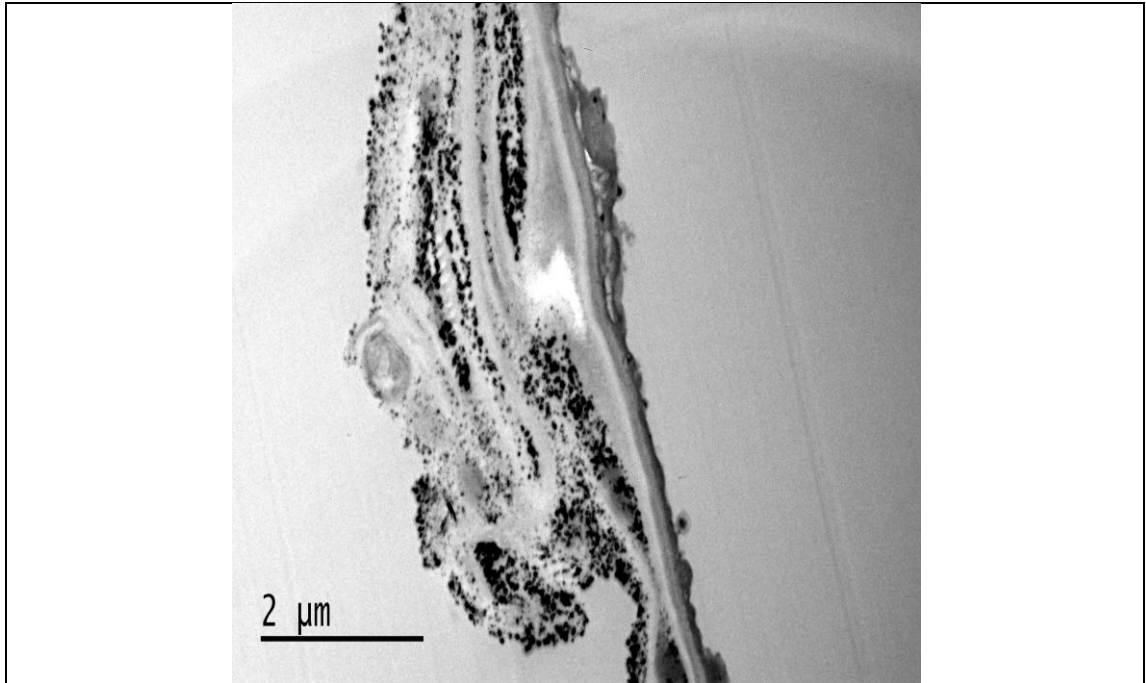


Fig 5.5.16 Cd+Cr+Pb Root semi-thin section

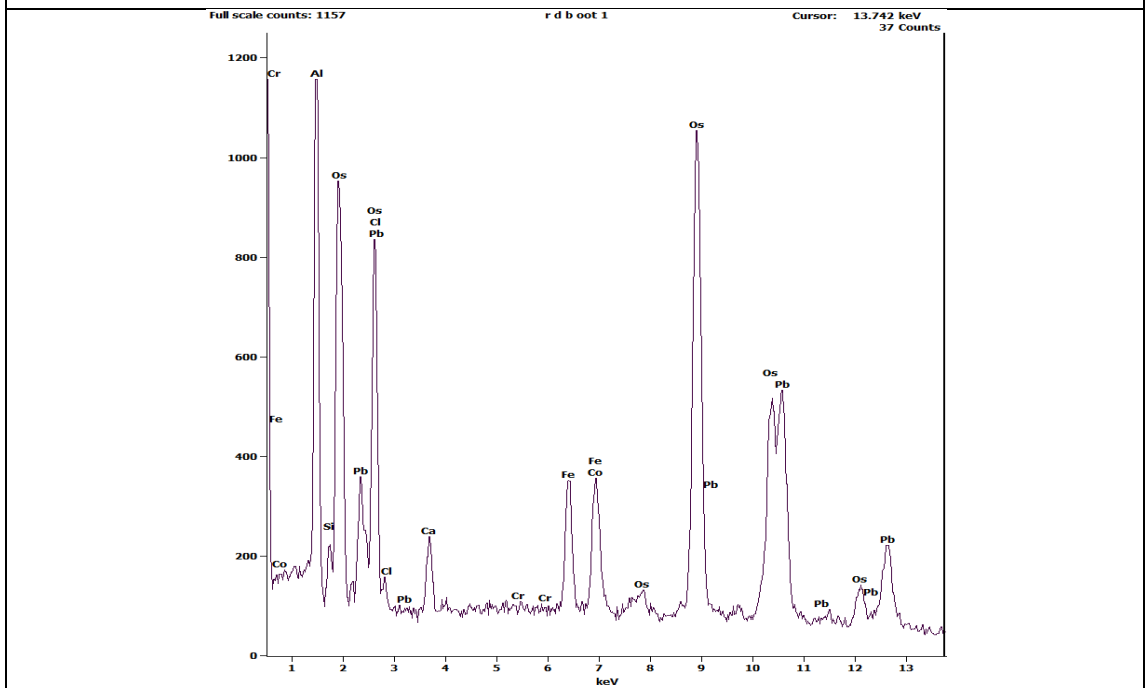


Chart 5.5.16 X-ray spectrum from Cd+Cr+Pb Root section

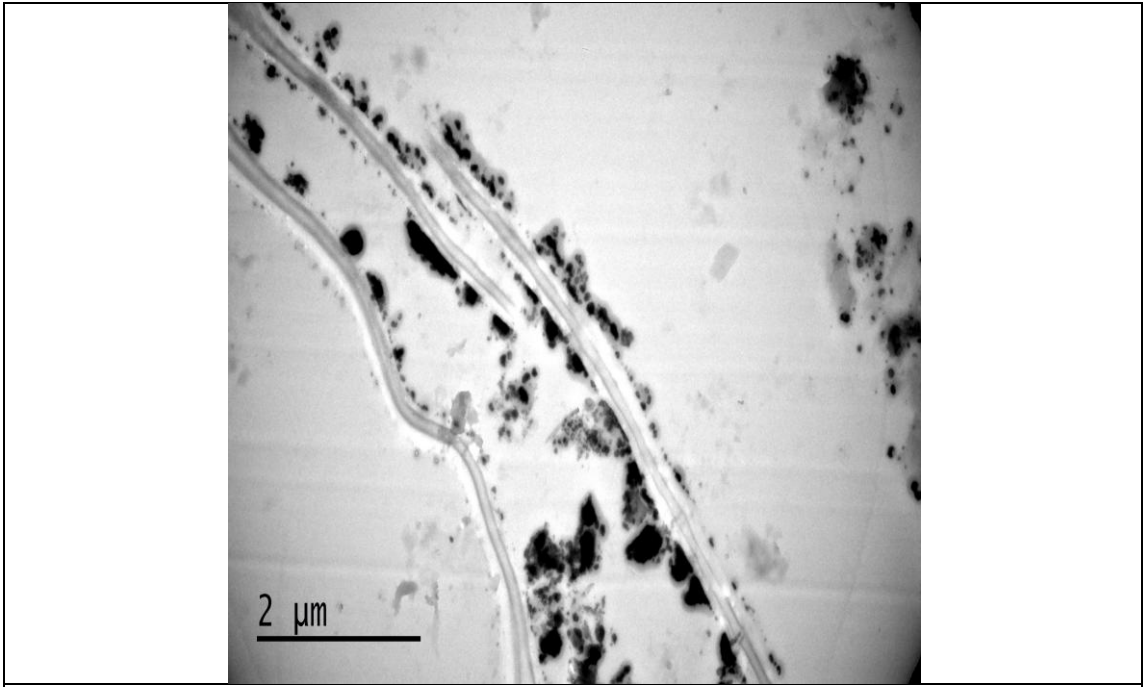


Fig 5.5.17 All Metals Root semi-thin section

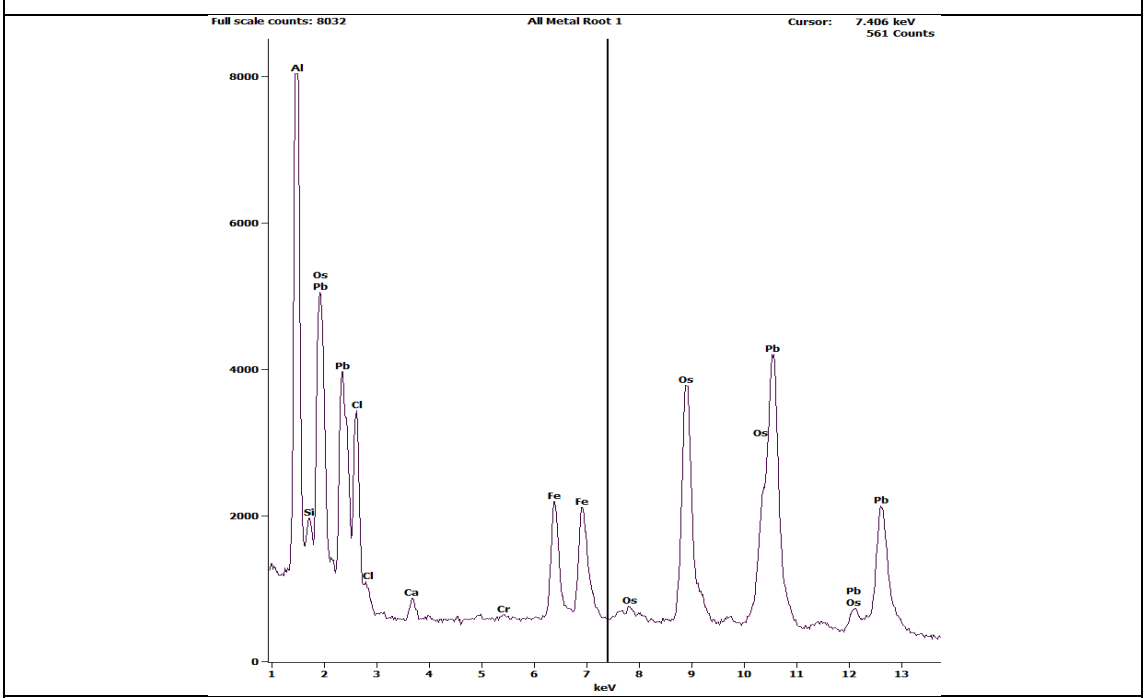


Chart 5.5.17 X-ray spectrum from ALL Metals Root section

5.6 Results from UV-Vis for nanoparticles synthesis

Figures 5.6.2-5 are the results of comparative potential of plant to synthesize silver nanoparticles after growing them on hydroponic nutrient media containing salts of cadmium, chromium, copper and lead and then leaching them in boiling water. 10ml of plant leachate was added to 90ml of a 1 millimolar solution of silver nitrate and the synthesis of silver nanoparticles was monitored by taking the absorbance at zero, 150 and 300 minutes. Figure 5.6.1 shows absorbance when only AgNO_3 in deionized water was taken as a control. Figures 5.6.2, 3, 4 and 5 are the results when 10 ml plant leachate was added to 90 ml of control solution. In figures 5.6.2, 3 and 4 two peaks are observed, one for the solution of silver nitrate similar to the control and other around 430 nm expected to be for silver nanoparticles. Figure 5.6.5 is similar to the control showing that there is no characteristic absorption peak when plant is grown on lead salt. Shift of peak from 230 to 440 nm, is maximum in copper followed by chromium and cadmium and no change in case of lead.

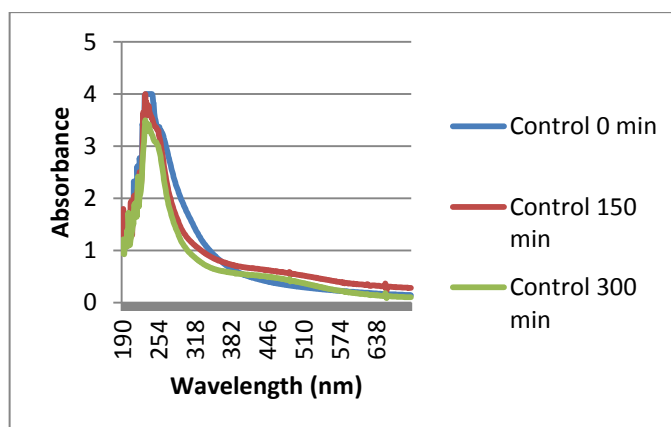


Figure 5.6.1: UV/Vis spectrum of a control leachate absorption sample containing only AgNO_3

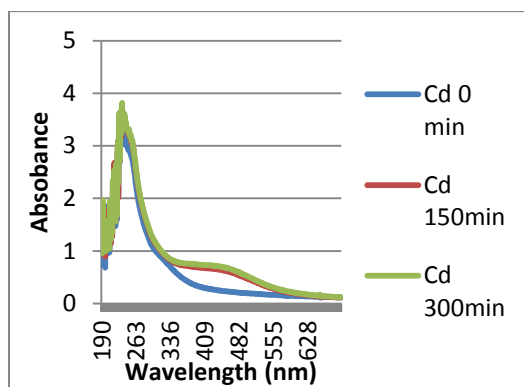


Figure 5.6.2: Cd leachate absorption graph

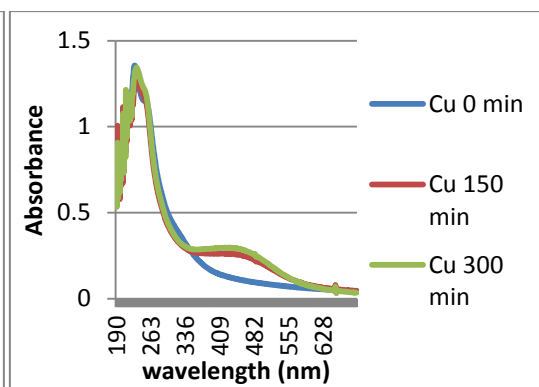


Figure 5.6.3: Cu leachate absorption graph

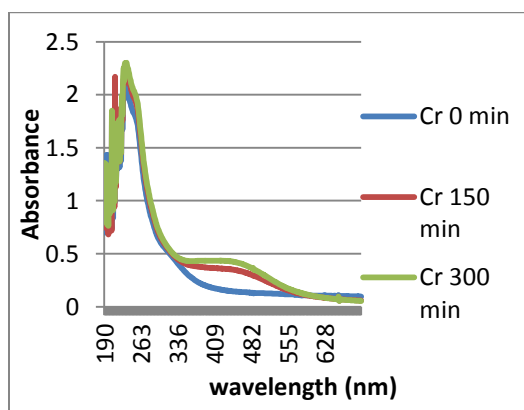


Figure 5.6.4: Cr leachate absorption graph

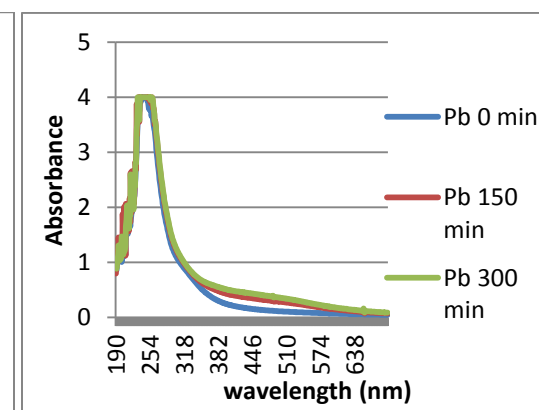


Figure 5.6.5: Pb leachate absorption graph

Figures 5.6.6-11, are the results of absorbance showing formation of silver nanoparticles when plants were grown on hydroponic nutrient media supplemented with two salts, of the four selected elements, in all possible combinations. Figure 5.6.6 shows decrease in absorbance at 230nm but there is not marked increase in absorbance at 430nm. In figure 5.6.7 there is visible peak at 430nm but no decrease in absorbance at 230nm. Both these confusing results are given by plants which have been grown on two salts which one of the salts is lead nitrate. The probable reason for this apparent anomaly is the rate of nanoparticle production was slower in the sample represented by Fig 5.6.6. Figure 5.6.11 is similar to figure 5.6.7 showing that

influence of Cd-Pb and Cu-Cr is similar on the ability of the leachate to manufacture silver nanoparticles. Figures 5.6.8, 9 and 10 are respectively, for the combinations of Cr-Cd, Cd-Pd and Cu-Cd.

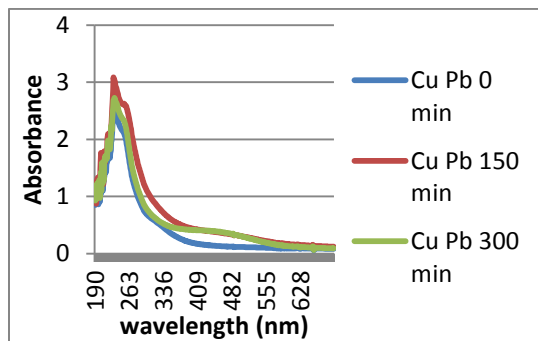


Figure 5.6.6: Cu-Pb leachate absorption graph

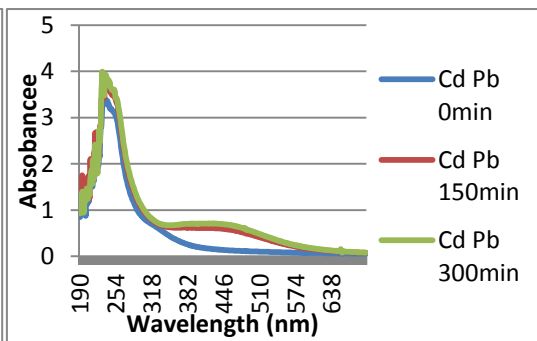


Figure 5.6.7: Cd-Pb leachate absorption graph

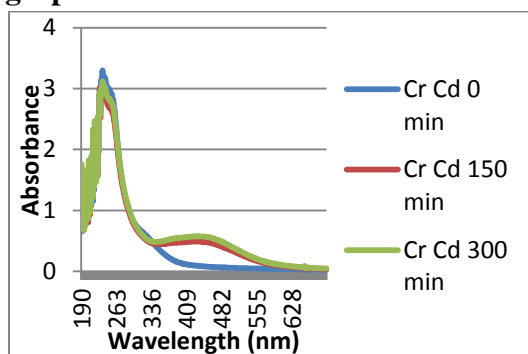


Figure 5.6.8: Cr-Cd leachate absorption graph

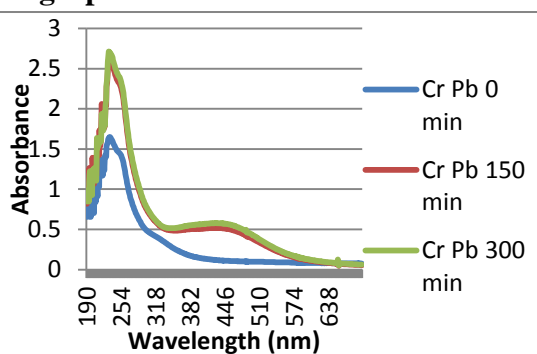


Figure 5.6.9: Cr-Pb leachate absorption graph

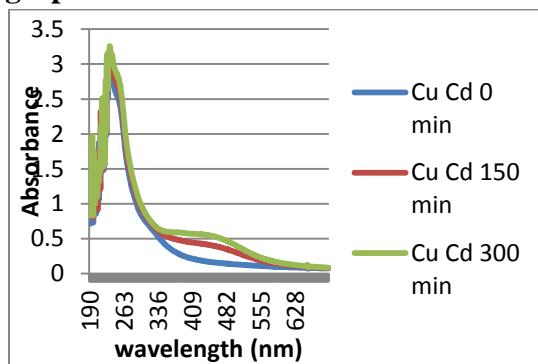


Figure 5.6.10: Cu-Cd leachate absorption graph

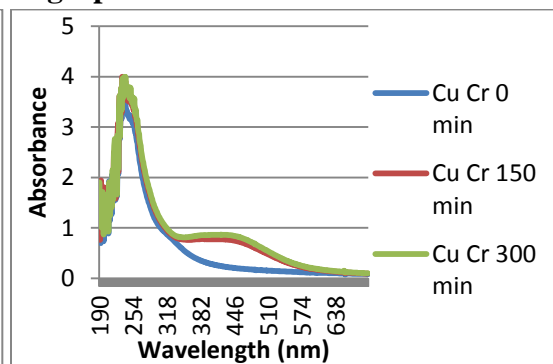


Figure 5.6.11: Cu-Cr leachate absorption graph

Figures 5.6.12-15 show potential of plant leachate to produce silver nanoparticles when salts of three metals were added simultaneously, to the nutrient medium used for

the growth of plants. Figures 5.6.12 and 5.6.13 show that Cu-Cd-Cr and Cu-Cr-Pb have positively influenced the plant with respect to their ability to form stable silver nanoparticles from the solution of silver nitrate. In figures 5.6.14 and 5.6.15 there is marked decrease in absorbance at 230 nm, showing decrease in the concentration of silver ions. However, there is negligible increase in absorbance at 430nm. These results indicate that Cu-Cd-Pb and Cd-Cr-Pb has influenced the physiology negatively. In figure 5.6.15 it can be seen that absorbance at 430nm is more at 150 min. than at 300 min.

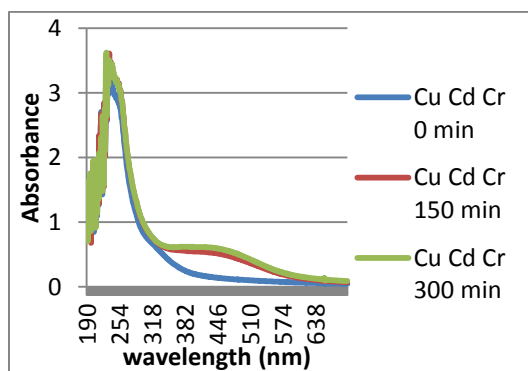


Figure 5.6.12: Cu-Cd-Cr leachate absorption graph

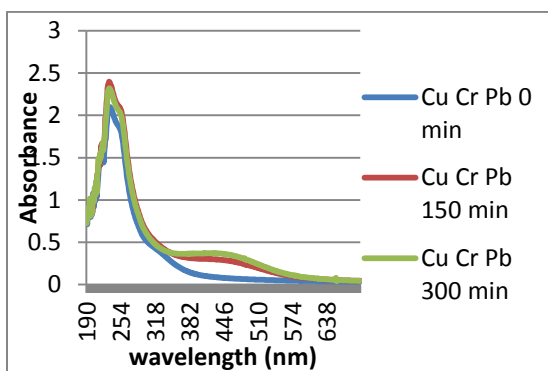


Figure 5.6.13: Cu-Cr-Pb leachate absorption graph

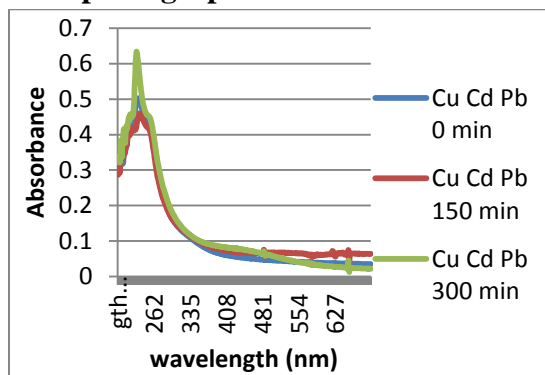


Figure 5.6.14: Cu-Cd-Pb leachate absorption graph

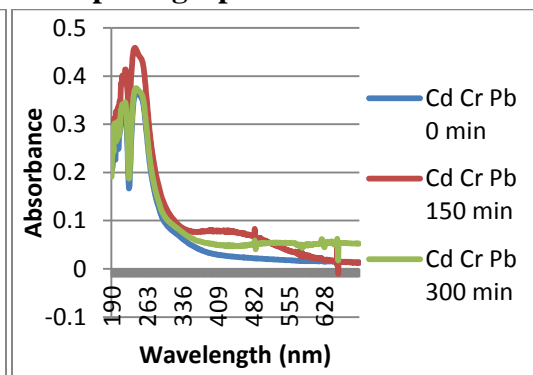


Figure 5.6.15: Cd-Cr-Pb leachate absorption graph

Figure 5.6.16 shows pattern of absorbance when all the four salts of Cu, Cd, Cr and Pb were added to the nutrient medium used for the growth of plants. There is a peak at approximately 440nm indicating conversion of silver ions to silver particles.

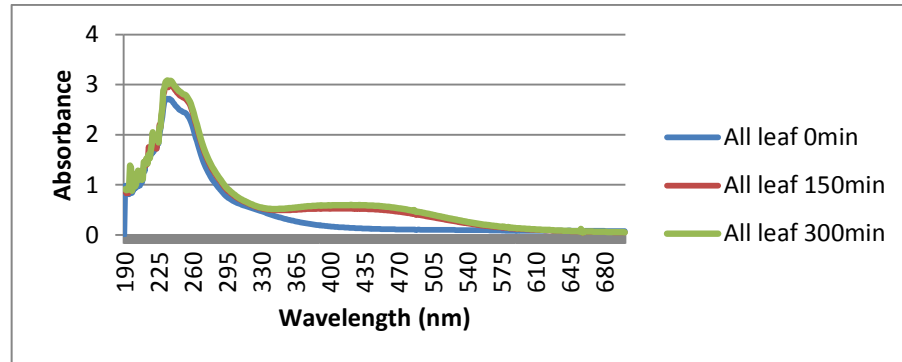


Figure 5.6.16: Cu-Cd-Cr-Pb leachate absorption graph

5.7 Comparison of metals extracts between *Phragmites australis* (PA) and *Zea Mays* (Zm)

Phragmites australis is one of the grass family and is categorised as a “C3” plant in its respiration characteristics whilst *Zea mays* is categorised as a “C4” plant which may be mono or dicotyledons.

C4 plants use a two stage process to generate carbohydrates from atmospheric CO₂; stage one involves the carboxylation of the CO₂ receptor phosphoenol pyruvate to C4 acids malate and or succinate in the mesophyll cells which is then transported to the sheath cells where, stage 2, the acids are decarboxylated to produce CO₂ which is converted into carbohydrates. The resulting C3 acids are transported back to the mesophyll to regenerate phosphoenol pyruvate. In C4 plants both stages are carried out in the light. C3 plants consist of the monocotyledons and include the grasses, reeds and cereals. Carboxylation of 3 molecules of the five carbon CO₂ receptor

ribulose 1-5 biphosphate forms 6 molecules of the three carbons, 3-phosphoglycerate, one of which is used for carbohydrate production and the other 5 used to regenerate ribulose 1-5 biphosphate (Bowyer and Leegood 1997, Taiz and Zeigler 1991).

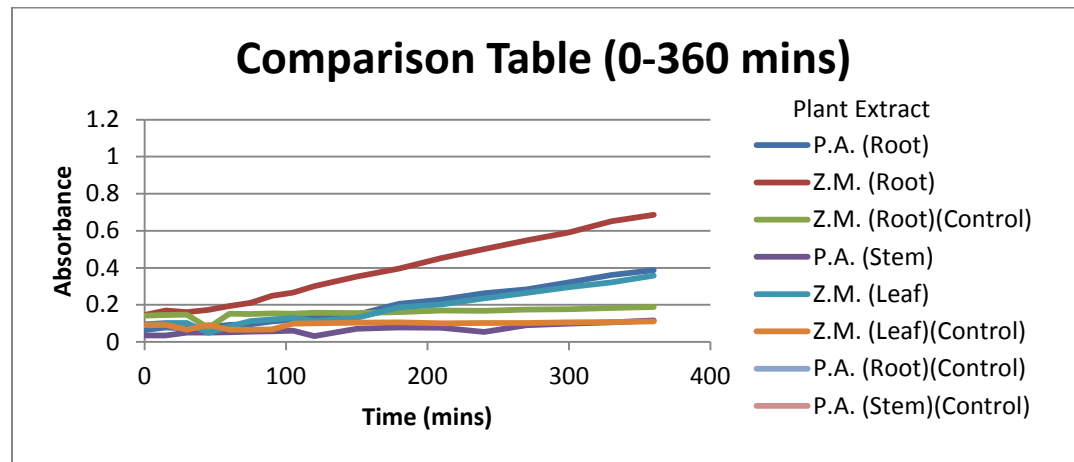


Fig 5.7.1 Comparison of nanoparticle production of PA v ZM at neutral pH from leachate

ZM Roots showed highest absorbance within above graphs which suggests they are most efficient at producing silver nanoparticles. PA stem showed more surprising results as the line for both graphs were nearer to the bottom of the graph, similar to the results of the control samples. PA root and ZM leaf were fairly similar shaped lines and consistent in both graphs, showing gradual absorbance increase overtime.

Zea Mays (ZM) root showed consistency and both had highest absorbance out of all the samples. ZM leaf was second highest but still gradually rose overtime, suggesting that ZM plants are efficient in producing silver nanoparticles compared to PA plants. The PA Stem showed much lower absorbance overtime for both graphs, and was the lowest in absorbance (apart from the control samples). PA root showed a little higher absorbance than PA leaf.

5.8 Effect of hydrogen ion concentration (pH) on the rate of production of nanoparticles

Three experiments were performed on leachates of PA and ZM roots and leaves at acid, neutral and alkaline pHs to determine whether this had an effect on the rate of nanoparticle production.

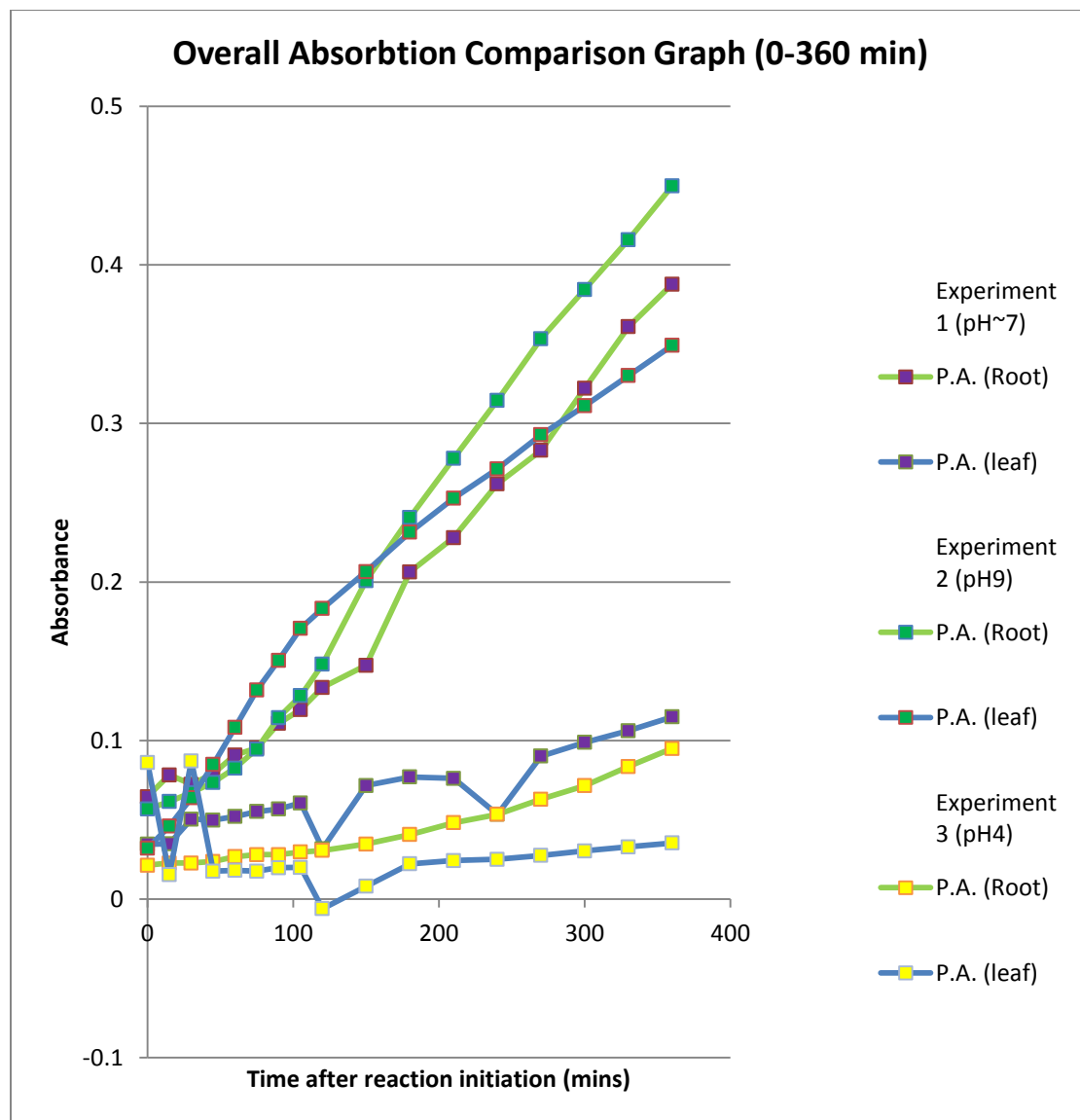


Fig 5.8.1 Graph of rate of nanoparticle production from leachate measured at 430nm, single replicates were used which were prepared from at least 10 plants per leachate

The results show that an alkaline pH is more conducive for nanoparticle production and also that the root leachate at pH7 was also faster at nanoparticle production. A pH of 3 did not show an enhanced rate of production and also the leaf extract at pH7.

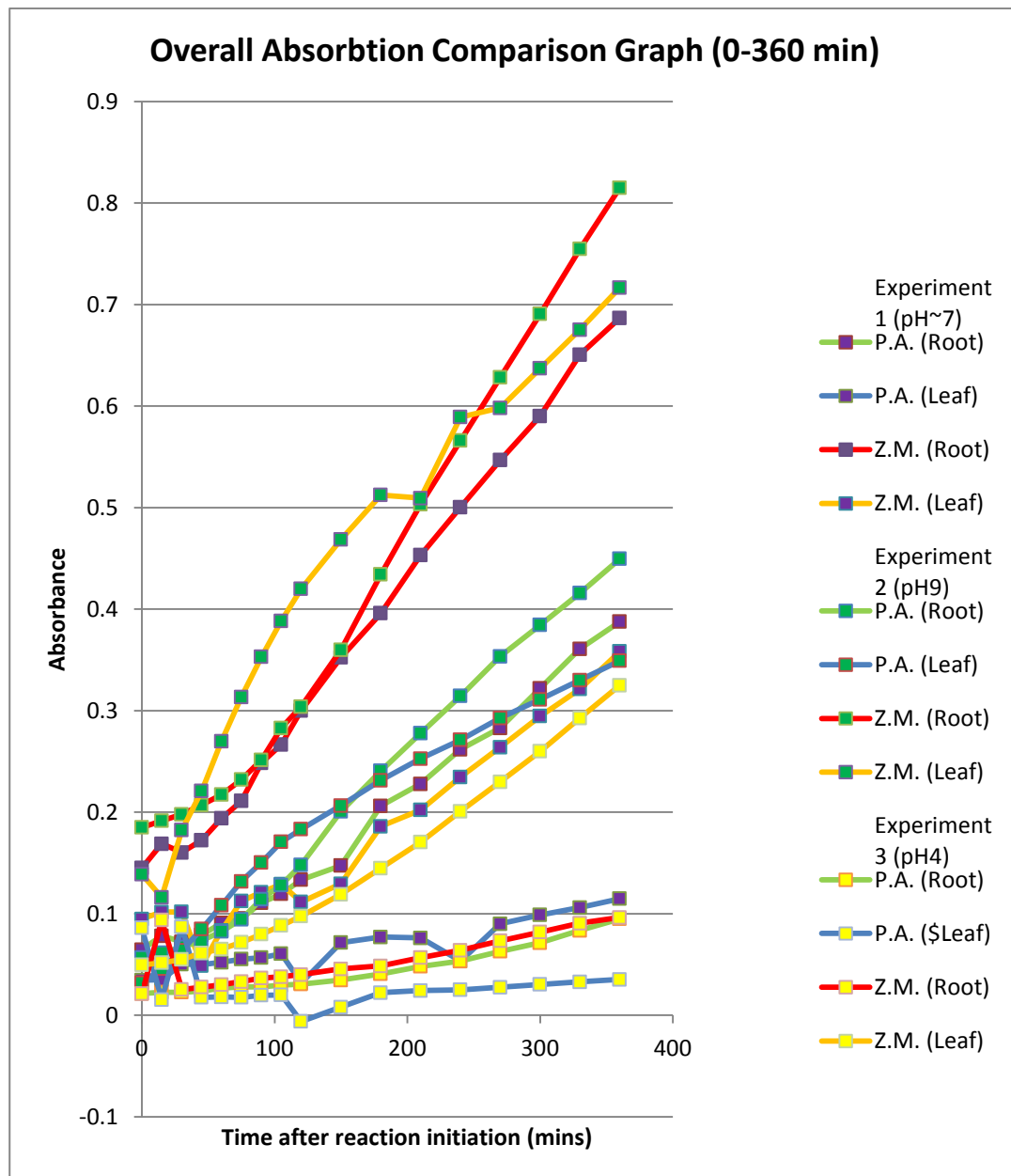


Fig 5.8.2 Comparison of the rate of nanoparticle production between PA and ZM plants

The results show that overall the rate of production for both leave and roots was fastest at an alkaine pH and slowest at an acid pH. Also ZM extract showed overall to be more efficient at nanoparticle production than PA. At alkaline conditions roots seemed to more efficient than leave.

5.9 Summary

Results from all laboratory tests performed by the author are displayed in this chapter and provides the following information as major findings in this thesis.

- Metal extraction capacity of PA plants for each metal combination in hydroponics
- Accumulation and location of absorbed metal in the plant roots, leaf or stem
- Effects of each metal on metal and on PA plants growth
- Capacity of roots, leaf and stem to absorb toxic metals
- The effects of Cd, Cr, Cu and Pb on the manufacture of silver nanoparticles
- Size of nanoparticles for each metal combination obtained for roots and leaf.
- The comparison of C3 and C4 plants for the manufacture of nanoparticles
- The effects of hydrogen ion concentration (pH) on the rate of nanoparticle production

These set of findings obtained from results will be analysed in the next chapter six.

Chapter 6 Discussion of the findings

6.1 Introduction

This chapter discusses the findings obtained from the results of laboratory tests carried out on reed plants and leached samples from the biomass. The main proposition of this thesis as defined in the first chapter was to remove heavy and toxic metals using reed plants and to assess the option of manufacturing nanoparticles as a “value added” step in the process of disposal. This research has shown that apart from copper, the accumulated metals were not extracted from the biomass and therefore the accumulated metals from the water would still have to be disposed through traditional methods such as landfill, incineration or ionization processes. This additional step may be used to offset some of the costs of these traditional methods which may benefit emerging economies due to the relatively low energy requirements and non-toxic bi-products.

The chapter discusses findings about making a case for phytoremediation using reed plants, metal absorption by reed plants, effects of metals, accumulation of metals in plants and manufacture of nanoparticles and their sizes.

6.2 Phytoremediation of metals using reed plants

The investigation in this thesis has applied ‘phytoextraction’ technique from a set of phytoremediation techniques (Salt et al, 1995; Jadia and Fulekar, 2009). It is found that it is possible to utilize reed plants as seen from the metal uptake tables in the

previous chapter. The technologies such as controlling release of contaminants, to accelerate breakdown of contaminants for remediation, extraction and treatment by ex-situ methods such as using carbon adsorption, microbes or air stripping are expensive as compared to the phytoremediation (Schnoor et al, 1995; Weyens et al, 2009; Jadia and Fulekar, 2009). In situ methods involve stimulation of anaerobic and aerobic microbial activities in the aquifer which also needs high capital expenditure and manpower as well as long term operating costs. Hence, efforts shall be made to develop more cost-effective approach such as phytoremediation using plants which are freely available. Phytoremediation is such a technology that utilizes plants and then the associated rhizosphere microorganisms to remove, transform, or contain toxic chemicals located in soils, sediments, ground water, surface water, and even the atmosphere. Currently, phytoremediation is used for treating many classes of contaminants including petroleum hydrocarbons, chlorinated solvents, pesticides, explosives, heavy metals and radionuclides, and landfill leachates. According to a report (Best et al., 1997), approximately 80% of the polluted ground waters are within 20 m of the surface. This suggests that a significant number of sites are potentially suitable for low cost phytoremediation applications. Utilisation of plants for remediating contaminated soils and ground waters is widely supported in the literature by Schnoor et al., 1995; Simonich and Hites, 1995; Watanabe, 1997; Chang and Corapcioglu, 1998 and Susarla et al, 2002. Therefore, findings of metal uptake by reed plants and literature support confirm validity of this proposition that reed plants promote by various processes the remediation of a wide range of toxic and heavy metals.

Post-phytoremediation disposal of biomass or removal of metal is of equal importance. For example, once the plants take up the metals, they can be removed and ashed. The ash can then be disposed of in a landfill or other contained area. If the metals are valuable, they can be recycled for their economic value (Wahla and Kirkham, 2008). Despite its advantages phytoremediation is in its infancy. The majority of the research for this stream is conducted in laboratories within controlled conditions for short periods of time. Thus, longitudinal studies with larger samples can prove the yield of the contributions made in this study. More extensive research under field conditions for longer durations is required. Same type of phytoremediation cannot be applied to every site. Also, interactions of plants can be affected by other living organisms such as insects, pests and pathogens, and exposure of plants to contaminants ultimately influencing the process of phytoremediation.

6.3 Metal absorption, uptake or extraction by reed plants

Cadmium

The graphs in the previous chapter for leaf and roots metal absorption at 2 and 4 weeks durations show that Cd alone is not absorbed much by plants whereas when Cd is combined with Cr and Pb is quadrupled in leaf and double in root. It is also evident that roots have more uptake than stem and leaf. Cd alone in roots have absorption rate of 15 to 20 ppm whereas the leaf has 1 to 2 ppm. This shows reed plants leaves are not able to extract Cd and their growth against Cd alone is negligible as all Cd is found in the roots. Root and leaf graphs show that addition of Cu with Cd reduces the uptake of Cd. The results show that reed plants are not Cd hyperaccumulator. Although, Cd uptake in plants is low, leachate sample testing showed all Cd cannot be extracted

from biomass. Thus, reed plants roots absorb Cd but release only small quantity of Cd. Jiang and Wang (2007) found Cadmium concentrations followed a gradient with the sequence: intercellular space > cell wall > vacuole > cytoplasm, indicating that most Cd was immobilized in the apoplast or sequestered into the vacuolar lumen. Our study is limited to the main parts of the plant and not on its organelles.

Chromium

The root and leaf uptake results show that Cr is not readily absorbed. The combination of Cr and Cu appears to be synergistic and at 4 weeks duration it is very high as seen from twin peaks in Cr graphs. It shows that Cd and Cu enable increase in Cr uptake.

Lead

As seen from Pb graphs, Pb+Cr combination is found at more than 88ppm at 4 weeks and 40 ppm at 2 weeks in stem and leaf. This is an exceptional case for Pb since rest of the absorptions are less than 7 ppm for stem and leaf. Also, this high absorption did not get extracted in the leached sample. It stayed in the plant as compared to Cd and Cr. Other combinations such as Pb+Cd, Pb+Cd+Cr and Pb+Cd+Cu are more evident in the roots. Roots have absorbed Pb and its combinations with other metals in the range of 20 to 89 ppm whereas leaching of these roots have released only 1 to 5 ppm. This proves that reed plants accumulates and holds the content of Pb and its combinations. Pb is very lethal and small amount of exposure can lead to severe diseases in human being. Thus, high accumulation and staying of Pb in reed plants is advantageous for the purposes of pollution removal from waterways.

Copper

Normally it is seen that single metals are absorbed in low quantities as compared to combinations of metals from all graphs of roots and stem and leaf. However, Cu and Pb alone are absorbed significantly by reed plants for example 19ppm and 59ppm respectively at 4 weeks duration.

The major difference between Pb, Cr, Cd group and Cu accumulation by reed plants is that all three Pb, Cr and Cd are accumulated high in roots but are not released back in their leach samples whereas Cu is extracted in the leachate sample. This increases the potential of Cu to be disposed of separately. Later in this chapter, the fate of the copper in the leachate solution will be discussed in the formation of nanoparticles. Thus, biomass formed with reed plants would still contain metals Pb, Cr, and Cd after leaching. Following table 6.2 shows the highest and lowest absorption cases of heavy and toxic metal combinations by reed plants. It reveals that Pb+Cr combination in roots of reed plants is the highest metal accumulated / absorbed.

Table 6.1 Reed plants capacity of metal phytoremediation

	Highest Metal absorbed In ppm	Lowest metal absorbed In ppm
At 2 Weeks		
Leaf	Pb+Cr – 88.75 Pb+Cd+Cr – 7.995	Cu – 0.265 Cu+Cr+Pb – 0.045
Roots	Pb+Cr – 88.75 Pb+Cd – 86.20	All metals (Cr) – 2.02 Cr+Cu+Pb – 2.264
At 4 Weeks		
Leaf	Pb+Cr – 40.755 Cd+Pb – 9.45	Cr+Pb – 0.285 Cr+Cd+Pb – 0.557
Roots	Pb+Cd – 88.40 Pb+Cd+Cu – 54.35	Cr+Cu+Cd – 1.926 Cr+Cu+Pb – 0.964
LEACHATE		
Leaf	Pb+Cd – 2.44 All metals (Pb) – 3.445	Cu – 0.01 Cd + Cu – 0.03
Roots	Cu - 22.945 Cu +Cr +Pb - 24.235	Cd+Cu+Cr – nil Pb+Cd+Cu – 0.005 All metals (Cd) – 0.01

(Source: Laboratory test results in chapter 4 * Denotes measured element)

Based on the metal absorption findings, it can be said for single metals that leaves of reed plants accumulates this group of metals in quantities: **Cu** <<< **Cr** < **Cd** << **Pb** and roots accumulates them in quantities: **Cr** < **Cu** < **Cd** < **Pb** in ascending order.

Metal uptake evidences by plants and methods of their determination by using FAAS are supported by literature. Heavy metals such as Cu, Fe, Co, Ni, Cd, Cr, Pb, Zn, and Mn concentrations in soils and in vegetable samples, i.e. lettuce (*Lactuca sativa* L.), parsley (*Petroselinum crispum*), dill (*Anethum graveolens*), and onion (*Allium cepa* L.) had also been reported in literature (Mullen, et al., 1996).

It is evident from the table 6.1 that combined metals are not significantly absorbed by reed plants in higher quantities. However, knowing reed plants capacity to extract more combined metals rather than single metal would enable further to analyse chemical reactivity and bioavailability of metals. Along with solid and gas phases water forms an environmental and biological reaction system wherein the water is polluted by combinations of heavy metals. This also allows extraction of known single metals in large quantities by using combinations of chemical extractants such as strong chelating agents or neutral salts (Baldantoni et al, 2008).

Factors affecting metal uptake and effects of metals in the solutions

There are three categories of factors affecting metal uptake in reed plants:

1. Metals and their effects; characteristics of reed plants and laboratory conditions commonly.
2. Accumulation of a given metal is a function of uptake capacity,
3. Intracellular binding (Clemens et al, 2002).

These conditions are affected by mobilization and uptake metals from water, compartmentalization and sequestration within the root of reed plants, efficiency of metal loading and transportation to stem and leaves, distribution between metal sinks in the aerial parts of the plants, sequestration and storage of metals in leaf cells. This each activity affects absorption, transportation and accumulation of metals in plants (Vymazal et al, 2007).

The comparative observations of all plants in hydroponic culture revealed that growth up to 2 weeks was at a normal growth rate for PA. Whereas during 2 to 4 weeks many plants colour changed to yellow and black with crumbled stems and falling leaves. This observation is clearly reflected in all stem and leaf graph as metal absorption reduces after 2 weeks which is probably due to the toxicity of the metals, especially chromium. Jiang and Wang, (2007) discuss the possibility that, when a plant initially grows, it absorbs more metal and once the metal is inside the cell its toxic effects may cause the reduction of metal within the plant.

6.4 Location of accumulated metals in reed plants

TEM results for metal location for selected metal combinations shown in section 5.5 of previous chapter revealed that most of the metal absorbed by plants was accumulated in roots and negligible amount of metals was found in PA leaves. This finding about metal accumulation in roots is equally supported by the FAAS results in section 5.2 for metal uptake wherein graphs show that roots have higher uptake of metal as compared to stem and leaves. In all experiments in which cadmium was included this metal was not found in the root or leaf samples analysed by TEM and EDX. In comparison copper, chromium and lead were found as discrete particles decorating the cell walls of the highly vacuolated cells of the roots, although not all of the target metals were found in all of the plant cells. This may be due to the non-uniform distribution of the metals in the cells but it is interesting that lead was found in all of the samples in which this metal was used. This suggests that lead is more easily absorbed by the plant roots than the other metals used in this study. Where lead is present in significant amounts in the roots phosphorus was also present

suggesting that the plant immobilises lead by converting it to insoluble lead phosphate. Unfortunately, phosphorus is partially overlapped by osmium in the X-ray spectrum which makes it difficult to assess whether phosphorus is present in all spectra where there are lead peaks. Further work to investigate the association of lead with phosphorus could be performed by omitting osmium from the fixation routine or by cryo-preservation. The absence of cadmium and chromium peaks in the X-ray spectra acquired from the semithin sections of roots and leaves may be due to the diffuse distribution of these metals in the plant tissue as discrete particles of these metals were not found.

6.5 Use of Reed plants

Reed plants can be used in two ways:

1. To extract polluting metals from water or soil,
2. To assess the toxicity of chemical substances.

In both cases germination of the plants is the important factor (Wang and Oyaizo, 2009). In this study however, germination was carried out separately before using PA plants in hydroponic culture. In this study germination of PA seeds was found to be difficult under laboratory conditions. Only by the use of a propagator and correct temperature and light condition did germination occur. It was also found that seeds extracted from intact seed plumes did not germinate successfully but commercially separated seeds gave better germination rates; these were used throughout the study. Although commercially germinated seedlings were available their seasonality was not suited to this study.

Bonnano and Giudice (2010) utilised reed plants as contamination indicators for heavy metals such as Cd, Cr, Cu, Hg, Mn, Ni, Pb, and Zn. They investigated whether the concentration of heavy metals in the roots, rhizomes, stems and leaves could indicate if plant organs could be characterized by differential accumulation, and to test the suitability of the various organs for heavy metal biomonitoring of water and soil. Results showed that metal concentrations in plant organs decreased in the order of root > rhizome _ leaf > stem. This study also found that Cd, Cr, Cu and Pb followed the same characteristics. These findings described the earlier sections have confirmed a set of reed plant characteristics within the context of phytoremediation techniques with multiple metals, the combination of which are prevalent in Saudi Arabia.

Wetlands have been widely used for waste water treatment with low cost and low energy requirement (Huett et al, 2005; Mungur et al, 1997). Wetlands plants such as reed plants can be deployed in the construction of artificial wetlands by implanting them in the local soil. Favourable characteristics of *Phragmites Australis* include adaptability to a wide range of climatic conditions, high biomass production and notably the ability of the root system to grow in low oxygen conditions and to tolerate levels of trace metals, organic acids and other breakdown products of organic matter that are toxic to most other plants (Ye et al., 1997). The major advantage as proven in this study is absorption and accumulation of heavy and toxic metals by reed plants (Mungur et al., 1997; Szymanowska et al., 1999). Roots of *P. australis* have been reported to accumulate Cr, Cu, Ni, Pb and Zn (Keller et al., 1998). Roots of PA plants are categorized as water roots and sediment roots (Damn et al, 2003). Experimental conditions are a major limiting factor in generalizing these results of metal uptake by

PA plants. It is not possible to faithfully replicate soil/water, weather conditions, oxygen content, toxicity of combined metals, existence of other chemical reactions and nutrients uptake from soil in hydroponic culture under laboratory conditions. However, this study attempted to regulate the temperature and metal concentration in solution. It would be desirable if PA plants could accumulate large amounts of toxic metals (hyperaccumulators) but the results of this study showed that reed plants do not meet the criteria of being a hyperaccumulator of any of these metals as in agreement with other authors (Roosens et al, 2003; January et al, 2008). Hyperaccumulator status is based on the ability to absorb and retain within the shoot (stem and leaves) one of the following metals at a minimum of: 10 mg⁻¹ of Zn or Mn, 1 mg⁻¹ of Ni, Co, Cr, Cu or Pb, 0.1 mg⁻¹ of As or Cd (Turgut et al., 2005), also that the most of the known hyperaccumulators are selective toward one metal (Kamnev and van der Lelie, 2000). The only concern in using *Phragmites australis* for waste water treatment is that if these plants are not harvested at the end of growing season, large amounts of decomposing leaf and stem waste is added to the aquatic system affecting the cycle of pollutants, such as heavy metals, present in the wastewater. Metal ions may become toxic to aquatic organisms. It is well known that these metals are toxic even if they are present in trace level concentrations, and their toxicity increases with increasing concentrations. A problem of transition metals is that they do not degrade through biological and chemical processes. Thus, reed plants can be used for large scale water treatment however; decomposing these plants is an important issue (Du Laing et al., 2006; Batty and Younger, 2007, Unamuno et al, 2007). This issue is addressed in the next section. Reed plants with absorbed metals were leached after microwave

digestion and mix with Silver nitrate to see if any of these metals – Cd, Cr, Pb or Cu would react with silver and manufacture metal nanoparticles.

Commercial reed beds

Application of reed plants, phytoremediation and nanoparticles manufacturing together promises a cost-effective way to remove heavy and toxic metals from water. The mechanistic approach to phytoremediation of water using conveyor production system can be developed to introduce commercial scale implementation of findings in this study (Adler et al, 2003)

6.6 Nanoparticles manufacturing from biomass

Leachate samples of leaf and roots showed us that more than 95% of the metals Cd, Cr and Pb were retained in the plants and the leachate solutions had very low metal concentrations. However, all combinations of leachate samples were separately mixed with AgNO_3 and the absorbance of the solutions monitored by UV/Vis spectroscopy to determine the rate of manufacture of nanoparticles. The resultant particle sizes of the silver nanoparticles were measured by a commercial Zetasizer and also by comparison of TEM images with a calibrated micron marker.

It was found that copper was present in the leachate and when mixed with AgNO_3 was incorporated into the silver nanoparticles. The other three metals were not leached in significant amounts therefore were not incorporated into the silver nanoparticles. The possibility to manufacture nanoparticles from leached biomass has three advantages:

1. To offset some of the cost of disposing of the biomass safely in the environment as leachates prepared from the biomass may be used to manufacture nanoparticles
2. Nanoparticles are utilized in industries such as electronics, biomedical, pharmaceutical, cosmetic, energy, environmental and catalysis.
3. Materials applications (Guzman et al, 2006).

Following table 6.2 shows the various sizes of metal combinations which is the smallest size in each category of leaf and roots leached samples. Nanoparticles are defined as particles having at least one useful dimension less than 100 nm.

Table 6.2 Nanoparticles manufactured from extracted metals

Size of Nanoparticles (nm) from metals in leaves		Size of Nanoparticles (nm) from metals in roots	
Cu	115.6	Cd	118.40
Cr	44.58	Cu	57.47
Cu-Cd	81.43	Cr-Pb	72.79
Cu-Cr	60.09	Cu-Cr	64.79
Cu-Cd-Pb	64.79	Cd-Cr-Pb	89.61
Cu-Cd-Cr	51.49	Cu-Cr-Pb	71.27
Cu-Cd-Cr-Pb	52.48	Cu-Cd-Cr-Pb	46.93

(Source: Zetasizer results)

The formation of nanoparticles was observed at 0, 150 and 300 minutes. From the TEM analysis of leached samples with the metal uptake, we observed that the morphology of the nanoparticles was typically spherical or rectangular in case of formation of copper/silver nanoparticles. The sizes of the nanoparticles ranged from 44.58 to 118.4nm; capping agents were not used to regulate their size, and this could

be investigated by further research. The images of nanoparticles in the previous chapter also reveal that metals are predominantly non-aggregated.

6.7 Validity of the research findings

The reliability of any research finding is a measure of consistency in the long term. For example, other researchers look for accuracy of measurement and replicability of findings for same investigations in different research contexts (Pallant, 2010). Whereas validity of the findings refers to measurements applied which in turn should predict, correlate and contribute to what they are measuring collectively. Therefore, the main validity checks are: is the methodology applied are reliable and relevant to the task; how novel the argument in the findings or proposition is; does refuting the existing literature by the researcher have any evidence; how much similarity or differentiation is there between existing and new findings. Therefore, following table 6.3 shows the support from the literature for materials and methods applied in this study which increases its validity. Table 6.4 shows findings of this research study and their support from the existing academic literature for similarity which again confirms the validity of these findings and further recommendations based on these findings.

A variety of methods have been used by researchers to localize heavy metals in plant tissues (Table 6.3). These include Autoradiography, Scanning electron microscopy (SEM); Transmission electron microscopy (TEM) and Scanning-transmission electron microscopy (STEM). Spectroscopy based techniques are also commonly used such as Electron energy loss spectroscopy (EELS); Laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS); SEM-energy dispersive X-ray

microanalysis (SEM-EDX); STEM-energy dispersive spectroscopy (STEM-EDS), Energy-dispersive X-ray microanalysis (EDX) and nanoscale secondary ion mass spectrometry. Other techniques include light microscopy, nuclear microscopy, micro X-ray fluorescence (μ -XRF).

Table 6.3 Validity of the methodology applied

Metal	Species	Tissue	Method	Supporting research done by
Cadmium (Cd)	<i>Echinochloa polystachya</i>	Leaves and stem	TEM, SEM, EDX	Solís-Domínguez et al. (2006)
Cadmium (Cd)	<i>Agrostis gigantea</i> and <i>Zea mays</i>	Root	TEM	Rausser & Ackerley (1987)
Copper (Cu)	<i>Eichhornia crassipes</i>	Root	STEM and X-ray microanalysis	Vesk et al. (1999)
Copper (Cu)	<i>Allium sativum</i>	Root	TEM and EELS	Liu & Kottke (2004)
Lead (Pb)	<i>Brassica juncea</i>	Root	STEM –energy dispersive spectroscopy, X-ray photoelectron spectroscopy	Meyers et al. (2008)
Lead (Pb)	<i>Brachiaria decumbens</i>	Root	TEM	Kopittke (2008)
Lead (Pb)	<i>Chloris gayana</i>	Root	TEM	Kopittke (2008)
Lead (Pb)	<i>Iris pseudacorus</i>	Root	TEM	Zhou et al.
Lead (Pb)	<i>Eichhornia crassipes</i>	Root	STEM and X-ray microanalysis	Vesk et al. (1999)

Table 6.4 Validity of the findings

Conceptual proposition of this study	Findings supporting the conceptual proposition in this study	Literature supporting the findings of this study
Phytoremediation characteristic of PA plants utilised to removal of Cd, Cr, Cu and Pb from water	PA plant roots absorb more metal than stem and leaf. Pb is the only metal to be heavily retained in the plants. Cu is fully extracted again through leaching and to use in nanoparticles manufacturing. Thus, Copper's complete removal is possible through phytoremediation.	Gardea-Torresday et al, 2005; Sasurla et al, 2002 Roman-Silva et al, 2003; January et al, 2008; Baldantoni et al, 2008; Bragato et al, 2006; Unamuno et al, 2007; Tylova et al, 2008
Potential of Nanoparticles manufacturing from extracted heavy and toxic metals for example Cu with AgNO ₃ in this study	Cu is dominant in manufacturing nanoparticles in combination with silver. All metal combinations of Cu, Cd, Cr and Pb were able to generate less than 100nm definitive nanoparticles promising industry usage.	Groning et al, 2002; Bali et al, 2006; Harris and Bali, 2008; Mohanpuria et al, 2008; Wang et al, 2009

(Source: Author)

6.8 Summary

In this study, the absorption and extraction of heavy metals using reed plants (PA plants) was studied. Major findings showed that:

1. Roots accumulate higher metal concentrations than leaves;
2. Virtually complete retention of Pb, Cr and Cd in plants when leached in boiling water.
3. Copper was released from plants on leaching.
4. Copper was incorporated into silver nanoparticles.
5. The ability of leachates prepared from the biomass to manufacture nanoparticles.

Based on these findings, it is recommended that PA plant:

1. Should be further investigated with other metals and metal combinations
2. Applied for commercial applications to treat metal contaminated water and soil
3. Further investigated for the manufacture of other pure metal and metal alloy nanoparticles from leached biomass
4. Be used as capping agents to moderate the particle size range of nanoparticles
5. Be used to investigate the possibility of recovering the retained metals to
 - A. possibly recycle these metals and
 - B. to reduce the environmental burden of disposal of contaminated waste

The results show that no important difference was observed in metal uptake function of reed plants except it was largely influenced by plant growth which was ultimately affected by toxic effects of metals on plant.

Other findings includes observations made during the laboratory tests and results obtained that

- Metal extraction capacity of PA plants depends on hydroponics conditions and plants
- Accumulation and location of absorbed metal in the plant roots, leaf or stem is affected by plant growth and internal plant characteristics.
- Capacity of PA roots to absorb metals is more than use of leaf and stem
- Cu has higher potential than Cd, Cr and Pb to manufacture nanoparticles

Chapter 7 Conclusion

7.1 Research summary

This chapter summarises the research study and explains the implication to theory and practice contributions, limitations and further research scope arising from this thesis. Based on the findings of this study, recommendations are made for use of *Phragmites australis* on a larger scale and in particular in Saudi Arabia where water resources are scarce. This is based not on the fact that *P. australis* is a hyperaccumulator but rather based on the observations that it will accumulate toxic metals and retain them in its roots and also that it is a prolific plant capable of generating large amounts of biomass.

The research design was implemented in the form of combination of literature analysis, industry data and laboratory experiments. Thus, the study deployed multiple data sources and multiple analysis techniques to test the proposition of using phytoremediation and manufacturing nanoparticles from contaminated biomass. To this effect, the major apparatus utilised were hydroponic cultivation, flame atomic absorption spectroscopy (FAAS), transmission electron microscopy (TEM) with energy dispersive X-ray analysis and ultraviolet/visible spectroscopy.

A surge in the non-destructive technologies such as rhizofiltration and phytoremediation to remove pollution from water and soil as compared to traditional technologies have prompted the researchers interest to study combination of heavy

and toxic metals to purify water using reed plants (*Phragmites australis*). These plants, whilst not hyperaccumulators, can be useful due to their metal absorption capacity and easy availability in the habitats around the world.

All metals singly, and in combination, were absorbed during the experiments in amounts above the water concentrations although growth was slow and chromium in particular proved to be extremely toxic resulting in chlorosis after a short exposure period.

The manufacture of silver nanoparticles was possible with contaminated biomass leachates and the experiments have proved that, with the exception of copper, no accumulated metals was released from the biomass and thus the nanoparticles formed were only of silver. The results obtained from the copper containing biomass, in that copper was released into the leachate and combined with the silver to form bi-metallic particles was interesting. This ability of the leachate to form pure silver or copper/silver nanoparticles may be the basis of an added value step in the remediation of waterways and, if this could be scaled to be commercially viable, may offset some of the costs of biomass disposal. Also, as the process of nanoparticle production is relatively low in cost and energy and may be beneficial to both developed and developing countries.

The major challenges during experiments were to replicate a growth environment for PA plants in hydroponic culture and to maintain the accuracy in laboratory procedures and use of apparatus. A specific recommendation such as use of PA plants on large

commercial scale for pollution removal from drinking water or waste water in Saudi Arabia is an additional output of this research study.

7.2 Implications for theory and practice

The research findings arising from this study have many theoretical and practical implications. These implications can either be positive or negative. The study provides set of important implications for researchers and pollution control practitioners. It is important to explore the possibilities of utilizing any positive implications for industrial usage.

One implication for research arises from finding that PA plants can absorb higher concentrations of certain metal combinations such as Pb+Cd, Pb+Cr and Pb+Cd+Cu. However, as the plants used for this study were young seedlings, these concentrations may be increased with larger PA plants under different research settings or natural conditions of habitat. Also, natural or artificial reed beds are possible which can uptake more metal as compared to indicative results obtained in this study.

Another implication is that Cu is able to combine with silver to manufacture bimetallic nanoparticles from the copper available in leachate samples obtained from the biomass containing the four metals used in the study. This may open new dimensions to nanoparticle manufacturing on a commercial scale and research themes. It is proved in this thesis that PA plants can absorb multiple toxic metals at the same time from solution thus PA plants can be further tested by researchers for other metals such as Arsenic or Mercury.

7.3 Contributions of this research

This thesis makes a theoretical contribution to the body of knowledge in terms of literature analyses, materials and methods, research findings and creating further research streams.

The first contribution is in terms of literature analyses about various heavy and toxic metal removal methods and making a case for phytoremediation technology. Secondly, it found that PA plants are not fully researched for their capacity to absorb such metals in combination and thus proposing to test PA plants for Cd, Cr, Cu and Pb combinations.

It is also found that Cu can be absorbed and disposed by manufacturing silver nanoparticles thus eliminating traditional methods such as landfill or incineration for removal of Cu. PA plants can be tested for other metals and these metals can be tested for their potential to manufacture nanoparticles. This combined theoretical and practical contribution as well, since nanoparticles have industrial usage and commercialization can be carried out.

PA plants can be used on large scale water pollution removal based on their tested and proven phytoremediation characteristic in this thesis. This is both a theoretical and practical contribution.

There are not many empirical studies found in the literature which have applied combined materials and methods used in this study such as PA plants, hydroponics,

FAAS, microwave digestion, TEM, Nanoparticles manufacturing centrifuge, UV-Vis and the use of the Zetasizer. This is again a contribution to theory for other researchers as a proven set of techniques.

7.4 Limitations

The research limitations of this study can be classified as methodological and theoretical limitations. The theoretical limitations include complexity of the subject matter and literature availability regarding use of PA plants for phytoremediation of waterways. The influence of theoretical limitations has been dealt with by accuracy and precision in carrying out laboratory tests and searching literature in all possible avenues such as industry data and academia.

The methodological limitation is that it is difficult to replicate a natural habitat setting in the greenhouse and in hydroponic cultivation. In addition to this, other factors such as evaporation from the hydroponic bath, loss of metal during microwave digestions or leachate preparation, pH, temperature and possible chemical reactions between metal compounds and the nutrient solution in the hydroponic baths can affect the metal absorption and their location in the PA plants. However, the precision of the analytical apparatus utilised, for example FAAS or TEM are unlikely to affect the results obtained, other than for the small uncertainties inherent in the techniques.

Thus, this study proves to be a guideline on the research stream of the use of PA plants and possible removal of Cu by manufacturing nanoparticles. The rest of the biomass containing the other three metals Cr, Cd and Pb has to be treated by

traditional methods of landfill or incineration or ionization as they do not have the potential of producing nanoparticles. These are methodological limitations.

Finally, the impact of such study may be felt in the long term when PA plants may be proven to be hyper-accumulator of other metals not yet researched or utilized on the industrial scale based on the findings in this thesis. Thus, generalizations of results can take place but it would take time before a sufficient body of knowledge is created around this research stream of pollution removal using phytoremediation through reed plants.

7.5 Recommendations

Findings in this thesis show that PA plants can absorb multiple metals, hence it is recommended to utilize them for pollution removal from water. As seen in the literature, PA plants can be useful to extract other metals and metals from soil also. Therefore, it is recommended to carry out research in detail and find out if PA plants are hyperaccumulators and of which metal.

Since, PA plants are commonly available and cause eutrophication in many habitats, which may or may not be desirable, it is recommended to develop their usage on a large scale pollution removal (or reeds bed as it is widely known in the trade) in suitable situations. This thesis concluded that Cu from biomass can be utilized to manufacture silver nanoparticles. Based on this finding, it is recommended to use this characteristic for industrial usage.

Phytoremediation is again proven in this study as a pollution removal technique and will add to the existing literature. Thus, it is recommended to use it over other expensive and traditional set of techniques to remove water pollution. Also, it is non-destructive technique which does not create other forms of pollution.

7.6 Future scope of research

As discussed in previous chapters, environmental pollution of ground water is a serious problem not only in industrialised countries but worldwide. New methods of remediation are needed to combat this problem but must be sympathetic to the environment and also be within the infrastructural capabilities of those countries. This research was performed at the request of the Government of the Kingdom of Saudi Arabia to answer a specific problem of metal pollution of scarce water resources. The research has shown that *P. australis* can be used as a metal sink and, as this is an ubiquitous plant, other countries can use this technology. The novel findings of this research in the manufacture of useful metallic nanoparticles by a low energy green route that is also environmentally friendly and adds value that may also be economically feasible for areas with little industrial resource. As the mechanism of metal absorption by this plant is not known, other fields of research in areas such as biochemistry, plant physiology and anatomy, biophysics and hydrodynamics may be used to study this phenomenon. Similarly, the mechanism of nanoparticle manufacture by the route described is also not understood even at a fundamental level which opens up further research fields such as chemistry and reaction kinetics.

Based on the findings of this research study, new research threads emerge based on methodological and conceptual context of this thesis creating scope for further research in the same domain.

Future research may focus on examining phytoaccumulation characteristics of PA plants for the same metals in different research setting and other metals in the same research setting. The set of materials and methodology adopted in this research was novel. Hence, it can be extended to research for other plants and metal combinations. There were limiting factors such as time available and number of hydroponic systems used for sampling. Thus, a horizontal study can be done with larger plant samples and more number of metal combinations to carry out statistical tests and test hypotheses.

This new research stream of incorporating metal by way of manufacturing nanoparticles is very promising for technological developments and futuristic application of biomass disposal on the large scale. This creates a whole new dimension in the industry wherein heavy and toxic metal pollution poses great environmental damage.

The same study can be replicated to test the findings and their validity creating a scope for usage of other materials and methods. For example microwave digestion conditions can be changed or there can be use of more advanced Zetasizer and TEM which can reveal detailed information about manufactured nanoparticles and metal accumulation in plants respectively.

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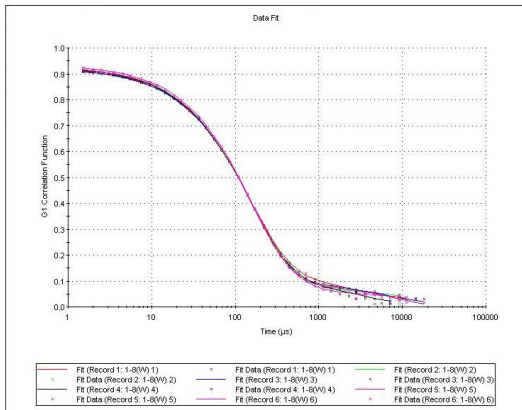
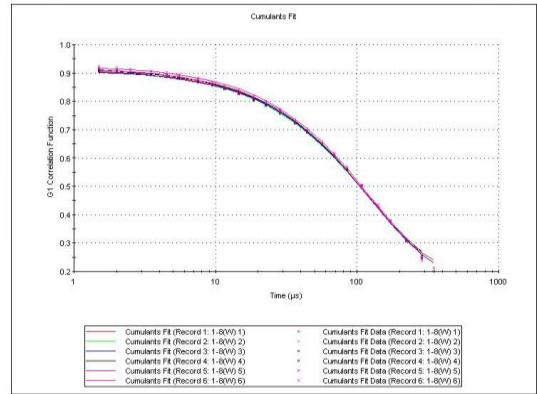
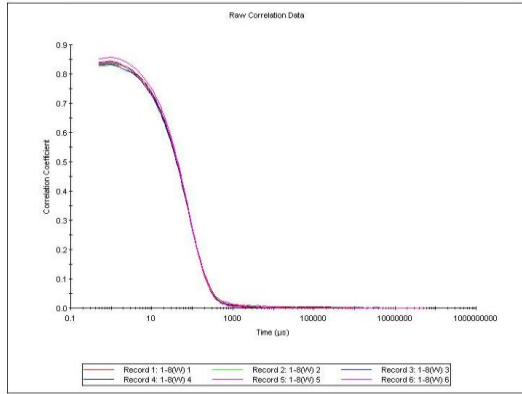
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Appendix A – Zetasizer results for metal nanoparticles

Following graphs were obtained for each metal combination. The examples are taken from all leaf and all roots.

ALL LEAF



Sample Name: 1-80V 6
SOP Name: mansettings nano
File Name: ALL Leaf.dts
Record Number: 6
Material RI: 1.33
Material Absorbance: 1.50
Dispersant Name: Water
Dispersant RI: 1.330
Viscosity (cP): 0.8872
Measurement Date and Time: 01 May 2012 13:05:38

Temperature (°C): 25.0
Count Rate (kcps): 170.2
Cell Description: Disposable sizing cuvette
Duration Used (s): 70
Measurement Position (mm): 1.25
Attenuator: 5

Z-Average (dLmm)	Peak 1:	Size (dLmm)	% Intensity	Width (dLmm)
52.48	60.34	60.34	96.3	35.73
PdI: 0.354	Peak 2:	4893	3.7	781.4
Intercept: 0.933	Peak 3:	0.000	0.0	0.000

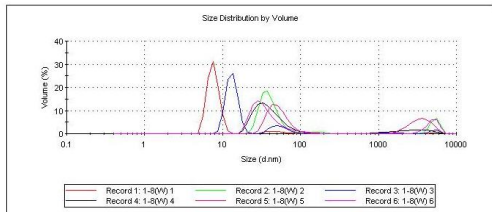
Result quality: Good

Sample Name: 1-80V 6
SOP Name: mansettings nano
File Name: ALL Leaf.dts
Record Number: 6
Material RI: 1.33
Material Absorbance: 1.50
Dispersant Name: Water
Dispersant RI: 1.330
Viscosity (cP): 0.8872
Measurement Date and Time: 01 May 2012 13:05:38

Temperature (°C): 25.0
Count Rate (kcps): 170.2
Cell Description: Disposable sizing cuvette
Duration Used (s): 70
Measurement Position (mm): 1.25
Attenuator: 5

Z-Average (dLmm)	Peak 1:	Size (dLmm)	% Volume	Width (dLmm)
52.48	38.45	38.45	80.4	21.73
PdI: 0.354	Peak 2:	4955	19.6	872.6
Intercept: 0.933	Peak 3:	0.000	0.0	0.000

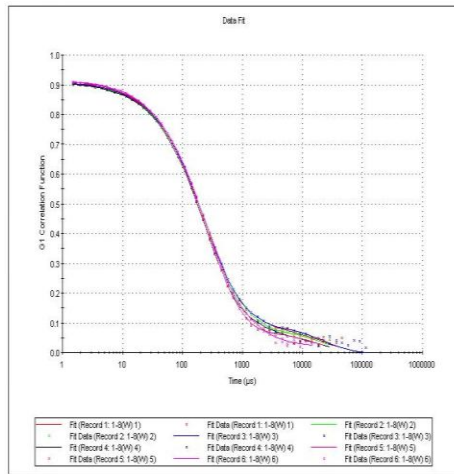
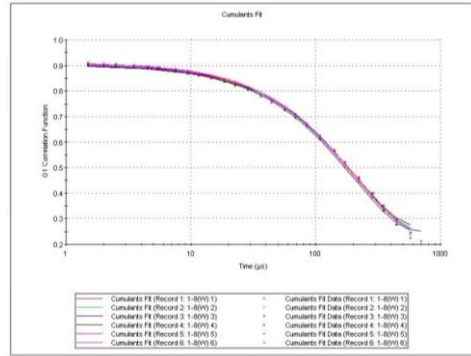
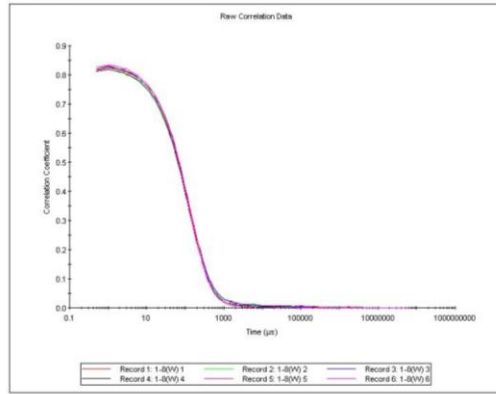
Result quality: Good



Sample Name: 1-80V 6
SOP Name: mansettings nano
File Name: ALL Leaf.dts
Record Number: 6
Measurement Date and Time: 01 May 2012 13:05:38
Temperature (°C): 25.0

RESULTS QUALITY CRITERIA

ALL ROOTS



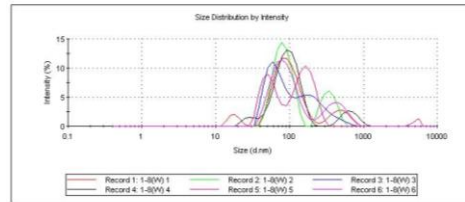
Sample Name: 1-8(W) 6
 SOP Name: mansellings nano
 File Name: ALL_Roots.dts
 Record Number: 6
 Material RE: 1.33
 Material Absorbance: 1.50

Dispersant Name: Water
 Dispersant RE: 1.330
 Viscosity (cP): 0.8972
 Measurement Date and Time: 01 May 2012 13:27:29

Temperature (°C): 25.0
 Duration Used (s): 70
 Count Rate (kcps): 209.7
 Measurement Position (mm): 1.25
 Cell Description: Disposable sizing cuvette
 Attenuator: 6

Z-Average (dLmm)	Peak 1:	% Intensity	Width (dLmm)
0.916	98.45	75.7	35.60
0.395	435.3	24.3	137.8
0.016	0.000	0.0	0.000

Result quality: **Good**



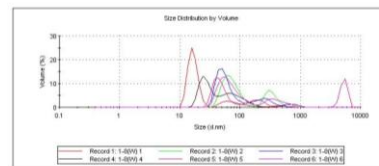
Sample Name: 1-8(W) 6
 SOP Name: mansellings nano
 File Name: ALL_Roots.dts
 Record Number: 6
 Material RE: 1.33
 Material Absorbance: 1.50

Dispersant Name: Water
 Dispersant RE: 1.330
 Viscosity (cP): 0.8972
 Measurement Date and Time: 01 May 2012 13:27:29

Temperature (°C): 25.0
 Duration Used (s): 70
 Count Rate (kcps): 209.7
 Measurement Position (mm): 1.25
 Cell Description: Disposable sizing cuvette
 Attenuator: 6

Z-Average (dLmm)	Peak 1:	% Volume	Width (dLmm)
0.916	69.81	77.8	30.39
0.395	404.1	22.2	152.5
0.016	0.000	0.0	0.000

Result quality: **Good**



Sample Name: 1-8(W) 6
 SOP Name: mansellings nano
 File Name: ALL_Roots.dts
 Record Number: 6
 Measurement Date and Time: 01 May 2012 13:27:29
 Temperature (°C): 25.0

RESULT MEETS QUALITY CRITERIA

