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ANALYSIS OF HEAVY METALS IN VEGETABLE AND AGRICULTURAL SOIL SAMPLES IN GEMIKONAGI AND DIPKARPAZ (NORTH CYPRUS)

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To God be the glory

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ABSTRACT

Heavy metal is a term used to describe metals that have a density greater than 5 g/cm3 and high relative atomic weight, very stable and non-biodegradable in the environment and are toxic at low concentrations both to plants, animals and human. Contamination of soil with heavy metals is mainly by anthropogenic activities. Plants take up heavy metals mainly by absorption through the roots from contaminated soil and also by their external parts such as leaves and fruits that are exposed to polluted environment. BCF-based studies revealed that the amount of heavy metal accumulation in vegetables is highest in leafy vegetables and least in fruit vegetables and moderate in tuber vegetables. Remediation of heavy metal contaminated soils can be done on-site or off-site but the off-site (excavation and disposal) remediation just remove the problem from one site and shift it to another site with dangers during the transportation of the soil to landfill disposal. The goal of this study was to investigate the levels of heavy metals in vegetable and agricultural soil sample thereby determining which plants is bio-accumulator by calculating the bio-concentration factor for each metal. The vegetable samples and soil samples were collected from Gemikonagi and Dipkarpaz. Gemikonagi is an ancient mining city and sea port but the mines have been abandoned with tailings. Dipkarpaz was used as the control area and the area has no history of mining activities. The distance between the two areas is approximately 150 km. The samples were analysed using inductively coupled plasma mass spectrometry and the heavy metal concentrations were determined. The results were compared using the SPSS statistical package. The order of heavy metal accumulation by the vegetables in Gemikonagi were malva > celery > cabbage > purple cabbage > broccoli > artichoke > lettuce > cauliflower > spring onion whereas in Dipkarpaz were malva > lettuce > celery > artichoke > cabbage > purple cabbage > spring onion. The vegetable samples from Gemikonagi had the highest mean concentration of heavy metals as compare to Dipkarpaz and the level in Gemikonagi (Malva 718.53 ppm) almost triple that in Dipkarpaz (Malva 240.47 ppm). There were 10 heavy metals which were analysed in the soil samples and these are the metals in increasing order of mean concentration in Gemikonagi Hg < Cd < Pb < Cu < As < Cr < Ni < Mg < Al < Fe and in Dipkarpaz Cu < As < Mg < Cr < Ni < Fe < Al. Among the detected metals in the soil samples, the concentration of Fe was the highest and the least concentration was Hg in the soil samples from Gemikonagi whereas in Dipkarpaz the highest was Al and the lowest was Cu. none of the vegetables were bioaccumulator as the highest BCF values were 0.2923 of Cu in Celery from Dipkarpaz and 0.2162 of Cd in artichoke from Gemikonagi. Majority of the heavy metals analysed were above the acceptable limit set by WHO and TSPCR which indicated that large amount of heavy metals is ingested through food.

Keywords: *Heavy metal, vegetables, agricultural soil, bioconcentration factor, North Cyprus*

1. INTRODUCTION

Heavy metal is a term used to describe metals that have a density greater than 5 g/cm^3 and high relative atomic weight, very stable and non-biodegradable in the environment and are toxic at low concentrations both to plants and animals and human (Alkas et al., 2017). Heavy metals of major concern are arsenic (As), nickel (Ni), cadmium (Cd), mercury (Hg), iron (Fe), manganese (Mn), cobalt (Co), chromium (Cr), lead (Pb), zinc (Zn) and copper (Cu). Heavy metals occurred ubiquitously in the environment, usually found in trace amount (ppb to ppm) in different matrices and their distribution is facilitated by natural and anthropogenic activities (Harmanescu et al., 2011 and Bortey et al., 2015). Vegetables are essential part in a healthy diet and health of humans. They have a wide variety of nutrients such as vitamins, dietary fibre, minerals, proteins and starch. Plants take up heavy metals mainly by absorption through the roots from contaminated soil and also by their external parts such as leaves and fruits that are exposed to polluted environment. Vegetable may also contain some amount of toxic elements (Pan et al., 2016 and Islam et al., 2007).

Contamination of soil with heavy metals is mainly by anthropogenic activities such as smelting, mining, application of fertilizer, pesticide and herbicides and irrigation with polluted water. Therefore, anthropogenic activities contribute more to the mobilisation of heavy metals which is a global problem (Sun et al., 2014).Table 1.1 shows the allowable levels of trace metals in agricultural soil in different countries. Plants inherently absorb pollutants from the environment and the chemical contents of plants can indicate the level of pollution when compared with the background values of unpolluted plants. The availability of metals in plants depends on a number of factors; clay minerals, soil pH, oxides, carbonates and organic matter (Angelova et al., 2010). Reports have shown that almost half of the average ingestion of cadmium, mercury and lead is linked to the consumption of fruits, vegetables and cereals. The uptake of metals by vegetables is through the roots by absorption from contaminated soil and also through the exposed parts of the vegetables in polluted air environment (Islam et al., 2007). The chemical form and binding characteristic of metals are key determining factor for the mobility and bioavailability of heavy metals in soil. Therefore it is of great importance for these forms and their characteristics to be studied. The sensitive sequential extraction procedure is used to understand and separate the geochemical fractions of heavy metals in soil and sediment and the fraction available to plants (Karak et al., 2010). In areas with high anthropogenic activities, heavy metals such as Lead, arsenic, copper, cadmium, mercury and chromium are environmental pollutants of significant interest as their accumulation in agricultural soil causes adverse effects on plant growth (phytotoxicity), food standard and environmental health (Islam et al., 2007).

The ability of vegetable plants to uptake and accumulate heavy metals differs widely with species. Lead is accumulated more in lettuce and onion while cadmium is more accumulated in spinach. The edible parts of leek, pak choi and carrots contain higher amount of cadmium than cucumber, tomato and radish. The accumulation of cadmium in vegetables is as follows; legumes < melons <alliums< roots <solanaceous< leafy vegetables (Zhou et al., 2016). The increase in soil and plant heavy metals can be attributed to the increased use of livestock and poultry manure and chemical fertilizers even though heavy metals are ubiquitous in the environment naturally (Jia et al., 2010). Diet is the main way by which the non-occupational population get exposed to trace element (Antoine et al., 2017).

Table 1.1: Regulatory concentrations (mg/kg) of toxic trace metals in agricultural soils of different countries (Liu et al., 2018)

Metals	EU	US	Australia	Taiwan	Canada	China
Cd	≤10	≤0.48	≤3	≤5	≤1.4	≤0.30 (pH≤7.5) ≤0.60 (pH>7.5)
Pb	≤200	≤200	≤300	≤500	≤70	≤250 (pH<6.5) ≤350 (pH>7.5)
Cr	≤200	≤11	≤50	≤250	≤64	≤250/150 (pH<6.5) ≤350/250 (pH>7.5)
Hg	≤2	≤1	≤1	≤2	≤6.6	≤0.30 (pH<6.5) ≤1.0 (pH>7.5)
Cu	≤150	≤270	≤100	≤200	≤63	≤50 (pH<6.5) ≤100 (pH≥6.5)
Zn	≤250	≤1100	≤200	≤600	≤200	≤200 (pH<6.5) ≤300 (pH>7.5)
Ni	≤100	≤72	≤60	≤200	≤50	≤40 (pH<6.5) ≤60 (pH>7.5)
As	≤50	≤0.11	≤20	≤60	≤12	≤30/40 (pH<6.5) ≤20/25 (pH>7.5)

Bio-concentration factor (BCF) is the ratio of the concentration of an element in plants to that in the surrounding soil, that is, heavy metal plant/soil ratio. BCF-based studies revealed that the amount of heavy metal accumulation in vegetables is highest in leafy vegetables and least in fruit vegetables and moderate in tuber vegetables. Based on the concentration of metals, Lead and Cadmium occur at high levels in leafy vegetables while Zinc concentration in tuber vegetables is the highest. The physio-chemical properties of soil such as texture, moisture, organic matter, pH and the cation exchange capacity (CEC) of soil greatly influence the form of the metals and their uptake into plants. Cadmium and Lead transfer from soil to plants is greatly influenced by soil pH and higher pH values decrease the bioavailability and toxicity of cadmium and lead. Air pollution can also enhance the accumulation of pollutants in the vegetable. BCF is a key quantitative indicator of plant contamination and the estimation of metal transfer from soil to plants by BCF has been seen in recent research (Chang et al., 2013). Plants with a bio-concentration factor more than 1 are termed hyper-accumulator and those with a factor below 1 are non-accumulators.

An adverse ecological effect of soil heavy metals contamination is a global environmental problem that needs intervention by both government and private agencies. The non-degradable nature of heavy metal is a major problem for the remediation of heavy metal polluted soil and the heavy metal pollution is a global problem that has attracted scholars from different parts of the world (Lai et al., 2013 and Xie et al., 2016). In order to minimise the availability of heavy metals in agricultural soils, agronomical practices must be applied such as soil organic matter management, pH modification, fertilizer management and also the type of vegetables and soil type. In areas where heavy metal pollution is not extensive, these techniques are suitable. The phytoremediation technique is used in highly polluted soils. This technique employs the use of metal accumulating plants to transport and concentrate heavy metals from polluted soil to the upper ground shoot which are harvested. This technique which uses higher plants to take up heavy metals from contaminated soils has recently been explored by many researchers (Islam et al., 2007).

The toxicity of heavy metal in plants, that is, phytotoxicity affects plant growth and development, causes oxidative stress and cytotoxic and genotoxic effects in plants. There are two primary routes of heavy metals exposure to humans; inhalation and ingestion. Ingestion through diet is the main route of exposure as we have seen over the years (the Minamata disease and itai itai in Japan). Chronic exposure to heavy metals in foodstuff may lead to interference of many biological and biochemical processes in the body of humans (Balkhair et al., 2016).

Heavy metals are toxic to humans and have the ability to accumulate in the body for a longer period of time. The different toxic metals exert different toxic effects:

arsenic cause angiosarcoma and skin cancer; long term exposure of cadmium cause liver and lungs acute toxicity, impair immune system function, induce osteotoxicity and nephrotoxicity; Lead on the other hand causes low intelligence quotient in Children, nephropathy, induce hypertension and cardiovascular disease (Zhou et al., 2016). Low levels of heavy metal exposure to animals have been carried out and their toxic effects observed with the first effects being trace element metabolism disruption. For example Cadmium replaces Calcium and causes osteodystrophy in the skeletal system; in the nervous system, Lead replaces calcium and impairs cognitive development (Angelova et al., 2010). Heavy metals can cause damages in lung, kidney, nervous tissues and skeletal system. Diseases associated to both short term and long term heavy metal exposure are coronary heart disease, cancers (renal, bladder and skin), gastrointestinal symptoms, reduced intellectual capacity and death in some cases (Maleki et al., 2014). Small amounts of methyl mercury can cause stillbirth or miscarriage (Yu et al., 2018).

Primarily, human exposure to heavy metals is through consumption of vegetables and fruits. It is therefore mandatory to analyse the level of heavy metal accumulation in crops such as vegetables from agricultural soil as vegetables are part of human daily diets. (Chang et al., 2013).

Geochemical studies revealed that Cyprus is naturally rich in copper and other metals and the distribution of metals is facilitated by anthropogenic activities such as mining, urbanization and agricultural activities. The region of Gemikonagi is known as an area of copper mining throughout the history of Cyprus and copper mining areas also contain some heavy metals such as cadmium, lead, chromium, mercury and arsenic. Mining activities in this region by Cyprus Mines Corporation (CMC) and other human activities facilitated the mobilisation of heavy metals to soil and water which are taken up by crops. The abandonment of the mining facility, mine waste and tailings of CMC without proper clean-up measures has let to contamination of the immediate area and other areas at large.

This study assessed the level of heavy metals in vegetables and agricultural soil in Gemikonagi region and Dipkarpaz, North Cyprus. Dipkarpaz with no mining and industrial activities which is located 150 km from Gemikonagi was used as a control area. Therefore the levels of heavy metals in soil and plants in Gemikonagi and Dipkarpaz were determined by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) and the results were compared using the SPSS statistical package.

The goal of this study is to investigate the levels of heavy metals in vegetable and agricultural soil sample thereby determining which plants is bio-accumulator by calculating the bio-concentration factor for each metal.

The results will be used to guide the farmers and entire population of North Cyprus for the choice of area (location) and type of crop for agriculture. This study will alert

the officials of Northern Cyprus if the levels of heavy metal are above the international accepted levels. The study is also a stepping stone for further studies to be carried out for the assessment of potential human health risk associated with food consumption using the Target Hazard Quotient (THQ).

2. HEAVY METALS AND THEIR PHYTOTOXIC POTENTIALS

Heavy metals occur naturally as ores in the earth crust with their respective relative abundance. They are naturally found in trace amounts and are non-biodegradable.

Table 2.1: Comparison of heavy metals levels in vegetables and soil with their maximum allowable limit

Heavy metals	Vegetables	Concentr. in soil (mg/kg)	Concentr. edible parts (mg/kg)	*Max. allow. limit	Posit. in earth's crust (ppm)[b]
Cd	*Lactuca sativa*	1.3	130	0.2	64 (0.11)
	Solanum lycopersicum	11.24	13		
	Agaricus bisporus	-	10		
	Brassica napus	1	6.0		
Pb	*Spinacia oleracea*	66.78	20	1	37 (14)
	Solanum aethiopicum	452	144		
	Brassica oleracea	2.58	49		
As	*Lactuca sativa*	5.83	14	0.15	55 (1.5)
	Oryza sativa	-	1.3		
Zn	*Zea mayis*	80	148	50	25 (75)
	Brassica juncea	190	201		
	Spinacia oleracea	124	84		
Ni	*Lactuca sativa*	1.11	48	0.2	24 (80)
	Cupressus empervirens	11.3	7.0		
Cu	*Zea mayis*	41	47	10	26 (50)
	Apium graveolens	46.85	11		
Cr	*Brassica oleracea*	12.78	24	1	22 (100)
	Solanum aethiopicum	256	65		
	Capsicum sinapsis	1.11	13		
Mn	*Allium cepa*	573	585	500	12 (950)
	Lactuca sativa	619	512		

*****EU standard 2006, FAOWHO/FAO 2007**,** [b]KennethBarbalace. Periodic Table 1995.Accessed on-line: /7/2018.https://EnvironmentalChemistry.com/yogi/periodic/

Most of the metals occurred as cations in soil with the exceptions of antimony, vanadium, molybdenum and arsenic occurring as oxyanions (Langmuir et al., 2003). Sources of heavy metal pollution are mining, smelting, fertilizers, pesticides, industrial waste and sewage sludge. Soil pollutions of these metals are harmful to plants and the environment (Ali et al., 2017). Due to the potential environmental risk posed by heavy metals, there is increased concern and this has prompted researchers to carry out large scale risk assessment (Cheyn et al., 2012). Table 2.1 shows the levels of heavy metal in some vegetables and in soil with their maximum allowable limit and the relative abundance of the metals.

Phytotoxicity is a toxic effect exerted on plants by chemicals and the effects can be summarised as plant growth inhibition (Table 2.2). Naturally, soil pH ranges from 4.0 to 9.0 in general environment but due to anthropogenic activities that contaminate soil with either acid or base, the pH can extend to the extreme from 2.0 to 11.0. Soils of this type are usually infertile and phytotoxic due the elements that are affected by extreme pH (Langmuir et al., 2003).

Table 2.2: Main effect of heavy metals in plants (Gardea-Torresdey et al., 2005)

Metals	Phytotoxic Effects
Cadmium	Decreases seed germination, lipid content, and plant growth; induces phytochelatins production.
Chromium	Decreases enzyme activity and plant growth; produces membrane damage, chlorosis and root damage.
Copper	Inhibits photosynthesis, plant growth and reproductive process; decreases thylakoid surface area.
Mercury	Decreases photosynthetic activity, water uptake and antioxidant enzymes; accumulates phenol and proline.
Nickel	Reduces seed germination, dry mass accumulation, protein production, chlorophylls and enzymes; increases free amino acids.
Lead	Reduces chlorophyll production and plant growth; increases superoxide dismutase.
Zinc	Seed germination; increases plant growth and ATP/chlorophyll ratio.

The presence of heavy metals in plants affect chlorophyll biosynthesis, cause lipid peroxidation, reduce respiration and also decrease the activities of antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), guaiacol peroxidase (POD). These antioxidant enzymes are widely used as biomarkers in soil polluted with heavy metals (Ali et al., 2017). Toxic effects of heavy metals are cellular metabolic arrest, cellular damage, and oxidative stress cause by reactive oxygen species (ROS) formation (Anjum et al., 2015). Certain heavy metals have inhibitory effects on the shoots (leaves and stems) and roots of plants and can also affect the germination process of plants.

Figure 2.1: Effects of heavy metals on plant leaves

Phytotoxicity caused by heavy metals can be explained using metal–soil physicochemical interactions such as (pH, organic matter, cation exchange capacity CEC, and texture) and plant uptake mechanism (active and passive transport across root membranes) as they greatly affect the form of heavy metals existence in soil and their phytoavailability (Ding et al., 2014). Example is the influence of CEC and pH of soil on Zn solubility (Kader et al., 2017). Heavy metals are readily available and mobile at low pH as they tend to adsorbed on the binding site of cation exchange of clay minerals and oxides through electrostatic bonds (Kim et al., 2015). Shahid et al. (2016), established a negative correlation between soil pH and heavy metal mobility and phytoavailability. The solubility, bioavailability and mobility of metals are high at lower pH and low at higher pH. Therefore desorption of metals in soil occurs at pH<7 and metals precipitate in soil at pH>8.Soils that are high in clay content have high cation exchange capacity, hence better cation adsorption. A report by Kim et al (2015) had shown that in a typical soil pH range, metal-organic complexes stability is in the following order; Cd, Ni and Zn have low stability while Cr, Pb and Cu have high stability. Reports have shown that Pb, Cr(3) and Ni are taken up by plants through passive uptake while Cu and Zn through active uptake.

Due to the phytotoxic potential of nickel, manganese, mercury, lead, chromium, and cadmium their respective plant uptake mechanism (phytoavailability), metal–soil physicochemical interactions, toxicity, relative abundance, and possible source of contamination are discussed in details below (Langmuir et al., 2003).

2.1 Mercury

Mercury exists in different forms; elemental Hg, organic Hg and inorganic Hg. The relative abundance of mercury in the continental crust is 400 mg/kg, in granite rocks is 80 mg/kg, and in shales 180400 mg/kg (Sasmaz et al., 2015). Mercury occurs in argillaceous sediments and fossil fuels and is very rare in the earth's crust. Organic Hg (II) complexes are dominant in soil and the mercury in soil is mostly bound to organic matter and clay minerals. Sources of mercury in the environment are anthropogenic and geogenic with anthropogenic emissions causing two thirds of the total Hg release in the environment. The major geogenic sources are forest fire, volcanic emissions, soil and water Hg volatilization, and weathering of rocks. All these sources of Hg pollution have different pathway/uptake mechanism into plants (Hlodák et al., 2016).

Worldwide data have shown that the mean hg concentration of top soils does not exceed 400 mg/kg and the highest mean mercury concentration was measured in Canada (400 mg/kg). The sources responsible for high mercury levels are Hg mining areas, base metal processing industries, volcanic areas and areas with fertilizers and pesticides application. The increased mobilization of mercury and higher levels in waters, air and soil are due to the many anthropogenic activities such as mining, smelting and agricultural practices. High quantity of Mercury is being emitted in many countries even though vigorous efforts have been made to minimize its release into the environment. Due to the high toxicity of Hg and its bio-accumulative character, it is considered a worldwide contaminant of concern and the different forms of mercury have different toxic potentials. The persistent nature of Hg and accumulation capacity makes it clean, very difficult and expensive (Sasmaz et al., 2015).

The main source of mercury exposure is through contaminated food consumption and higher levels of methyl mercury are being found in fish and aquatic invertebrates because of the ability of mercury bioaccumulation and bio-magnification. There are many reports on chronic mercury exposure to animals in which adverse effects were observed in fertility but there is little knowledge about the reproductive toxicity in humans though it is known to be neurotoxic. Epidemiological data among women that are exposed to mercury occupationally revealed menstrual cycle abnormalities. In animals, mercury exposure induced stillbirth, ovulation inhibition, infertility and congenital malformation. According to

the observations, it has been suggested that mercury have an impact on reproduction in occupationally exposed women.

The three soluble forms of mercury that exist in soil are Hg^0, Hg^{1+}, and Hg^{2+}. The latter 2 forms exist in oxidized form at low pH and Hg^{2+} is unstable at normal environmental condition and it changes to Hg^0, Hg^{1+}. Aerobic bacteria also convert soil mercury into methyl or dimethyl mercury in the process of methylation (Tangahu et al., 2011). Mercury mobility in soil depends on chemical and biological degradation of organomercury compounds and dissolution processes (Kabata et al., 2001). It has been reported that the bioavailability and phytotoxicity of Hg is lower in age soils. Organic matter contains the Cl-, OH-, and S2 functional groups that have a high affinity for mercuric compounds and hence form stable and strong complexes.

Mercury accumulation in plants is related to the characteristics of soil and also the concentration of Hg in soil. Less Hg is taken up by plants when the soil pH is high, accumulated salts and surplus lime in soil. The soil organic matter also plays an important role in mercury uptake. The concentration of mercury in plants is directly proportional to that in soil as it has been reported that when the only source of the metal was soil, the concentration was high (Sasmaz et al., 2015). Mercury accumulation is more in the plant roots and the roots take up mobilized Hg easily and the plant roots act as a barrier for mercury not to be translocated to the shoots. The bioavailability of Hg in soil increases with low soil organic matter, and oxidative weathering or enhanced microbial activity (Hlodák et al., 2016).

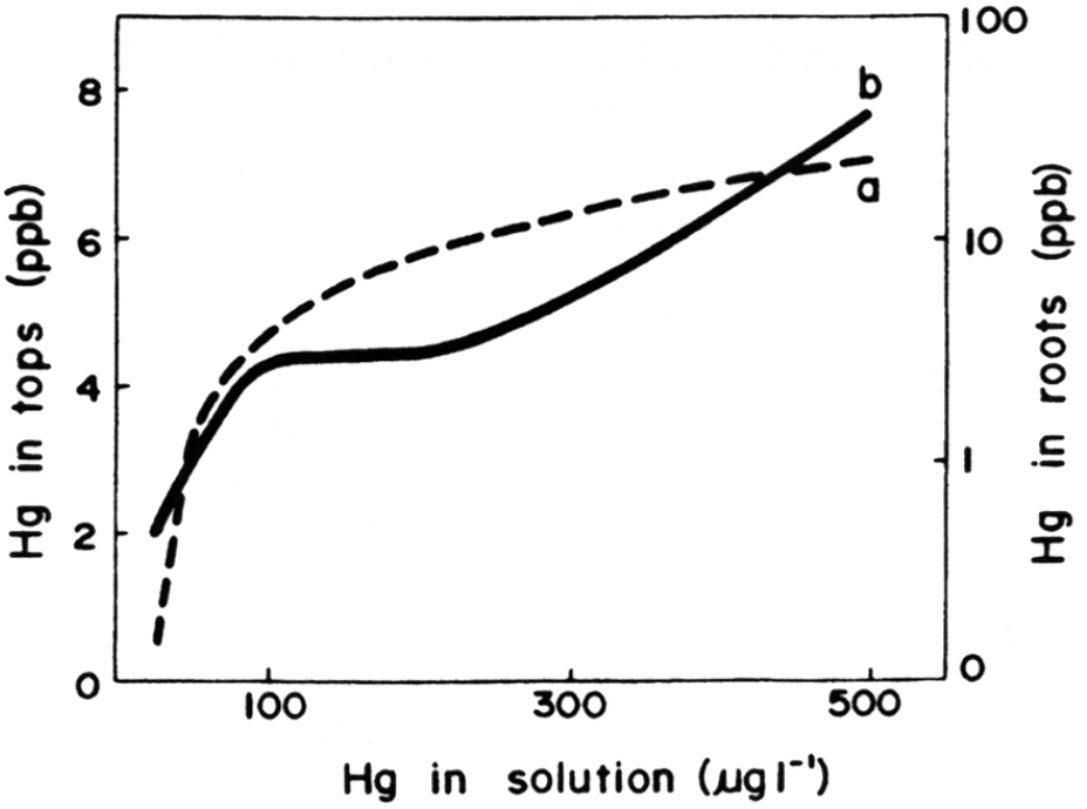


Figure 2.2: Uptake of Hg by 7-day-old oat seedlings from the culture solution of HgNO3 concentration. (a) Tops; (b) roots. (Kabata et al., 2001)

Vegetables and fruits have a background level of mercury that vary from 2.6-86 ppb (DW) and 0.6-70 ppb (FW). Increasing Hg contents in soil, leads to an increase in plant mercury contents. Plant roots absorb mercury easily and the Hg is translocated to other parts of the plants. The roots have been reported to accumulate the highest amount of Hg as compare to the little amount in the leaves (Kabata et al., 2001, Hlodak et al., 2015). High mercury concentrations have been observed in carrots, onions, garlic, radish, beets, parsnips, turnips and other root vegetables. The mercury accumulated by plant roots also inhibit potassium ion uptake. The translocation of mercury occurs in different tissues in plant; from leaves to grains in rice plant, leaves to fruits and also from seeds to the first generation seeds of wheats/peas treated with fungicides containing mercury (Kabata et al., 2001). Vegetables accumulate higher amount of Hg than fruits and grains and different vegetables have different capacity to accumulate Hg; Parsley and Lettuce Hg concentration greater than potatoes, cucumbers and tomatoes Hg concentration (Sasmaz et al., 2015). Lettuce, carrots, mushrooms and lichens take up higher concentration of mercury than other plants growing on the same area (Kabata et al., 2001).

Mercury phytotoxic effects are oxidative stress initiated by reactive oxygen species and free radical compound induced by mercury and also affect the morphology and physiology of plants (decreased uptake of essential elements; growth inhibition in root and shoot; inhibition of photosynthetic pigment synthesis). The levels of superoxide dismutase, peroxidase and catalase antioxidant enzymes are also increased in the presence of mercury in plants. Mercury interferes with the electron transport in chloroplast and mitochondria thereby affecting oxidative metabolism and photosynthesis. Hg also inhibits aquaporins and reduces the uptake of water by plants (Tangahu et al., 2011). Phytotoxicity of mercury can be summarised as (a) reduction in nutrient uptake and plant growth inhibition; (b) inhibit photosynthesis; (c) induce genotoxic effects; (d) affects antioxidative systems. Other researchers also reported that small concentration of mercury in plants can induce oxidative stress and lipid peroxidation which increase the activity of antioxidant enzymes (glutathione, peroxidase, ascorbate peroxidase, superoxide dismutase and glutathione reductase) (Kumar et al., 2013).

2.2 Cadmium

Cadmium is a rare element in the earth's crust and it is the 65th most abundance element in the earth's crust. It was discovered by Stromeyer and Hermann in Germany in 1817 as a by-product of Zn smelting. The elevated soil cadmium concentration is as a result of Zinc mining, smelting, application of insecticides, fertilizers and pesticides, and also sewage sludge application. Phosphate-based fertilizers and fertilizers made from sediments of sea bed contain high concentrations of cadmium. Cadmium has a high mobility in soil and is easily taken up by plants. Cadmium natural concentration in soil range from 0.07-1.1 mg/kg globally and cadmium is phytotoxic above 10 mg Cd/kg soil. The total cadmium concentration by FOREGS in agricultural soil in Europe is between 0.06-0.6 mg/kg (Shahid et al., 2016). The presence of cadmium in the environment is of high concern. Cadmium is found in very low concentration in soils and raises concern when found in agricultural soil. Cadmium is an ecotoxic chemical. Sources of cadmium pollution to the environment are metal mining, smelting operations, fertilizers and pesticides application and industrial activities and these activities lead to elevated levels of cadmium in the environment (Lamb et al., 2016).

In agricultural soils, cadmium pollution is the most wide spread as compare to other heavy metals due to anthropogenic activities such as Sewage sludge and phosphate fertilizers. The cadmium concentrations in urban soil in china range from 0.11 to 8.59 mg/kg (Zhao et al., 2017). Plant cadmium concentration is high in polluted environment because cadmium is highly phytoavailable both from soil and air (Kabata et al., 2001). Cadmium exists as cation at normal environmental pH but

becomes cadmium hydroxyl species when pH is increased. In solution studies, as the Cd hydroxyl species increase in the soil solution, the uptake by plant roots also increases which is translocated to the shoots and cause toxicity in plants. Therefore the uptake of cadmium is increase with increasing pH which may be attributed to sorption to external cells and the changes that occur in the surface of the apoplast. Cadmium absorption by plant roots is affected by the presence of humic acid (Lamb et al., 2016). The phytoavailability of cadmium is influence by many factors both soil physio-chemical properties and the physiology of plant. The soil properties are soil particle size, cation exchange capacity, temperature and pH whereas the physiological characteristics of plant are root exudation and transpiration rate, and surface area of root. Many species of plant accumulate cadmium in the roots and the amount translocated to the shoots is very little. The phytoavailability of Cd is directly proportional to the total Cd concentration in soil because of the different forms and distribution of Cd in soil. Cd can either be free or adsorbed and this affects the amount available for uptake. The readily availability of cadmium to plants is due to the fact that they are predominantly bound to the exchangeable solid phase which is easily release into soil solution. Cd^{2+} ion is the predominant form of cadmium in soil. The uptake of cadmium by plant is mainly through pore water (Shahid et al., 2016).

The various forms of Cd in soil are control by formation of Cd-ligand complex, precipitation/dissolution, and adsorption/desorption reactions. These reactions are affected by cation exchange capacity, soil texture, metal burdens, soil pH, temperature and competing cations. The pathway of cadmium entry into plant is not specific. It occurs through root uptake by specific and non-specific essential elements transporters. It has been shown that essential elements such as Ca^{2+}, Zn^{2+}, Fe^{2+}, Cu^{2+} and Mg^{2+} inhibit the uptake of Cd due to the competition for transporters (Shahid et al., 2016). Cadmium is taken up by membrane transporters readily and has a relatively high mobility in soil (Zhao et al., 2017).

The uptake of cadmium is affected by many plant and soil factors. Cadmium is easily absorbed by the leaves and roots though it is a non-essential metal. A global research carried out in 30 countries shows that plant cadmium is a function of soil cadmium. The mechanism of Cd^{2+} uptake is not fully known but it is likely to be transported by the same mechanism for Zinc translocation (Kabata et al., 2001). The figure (2.3) below summarised the cycle of cadmium.

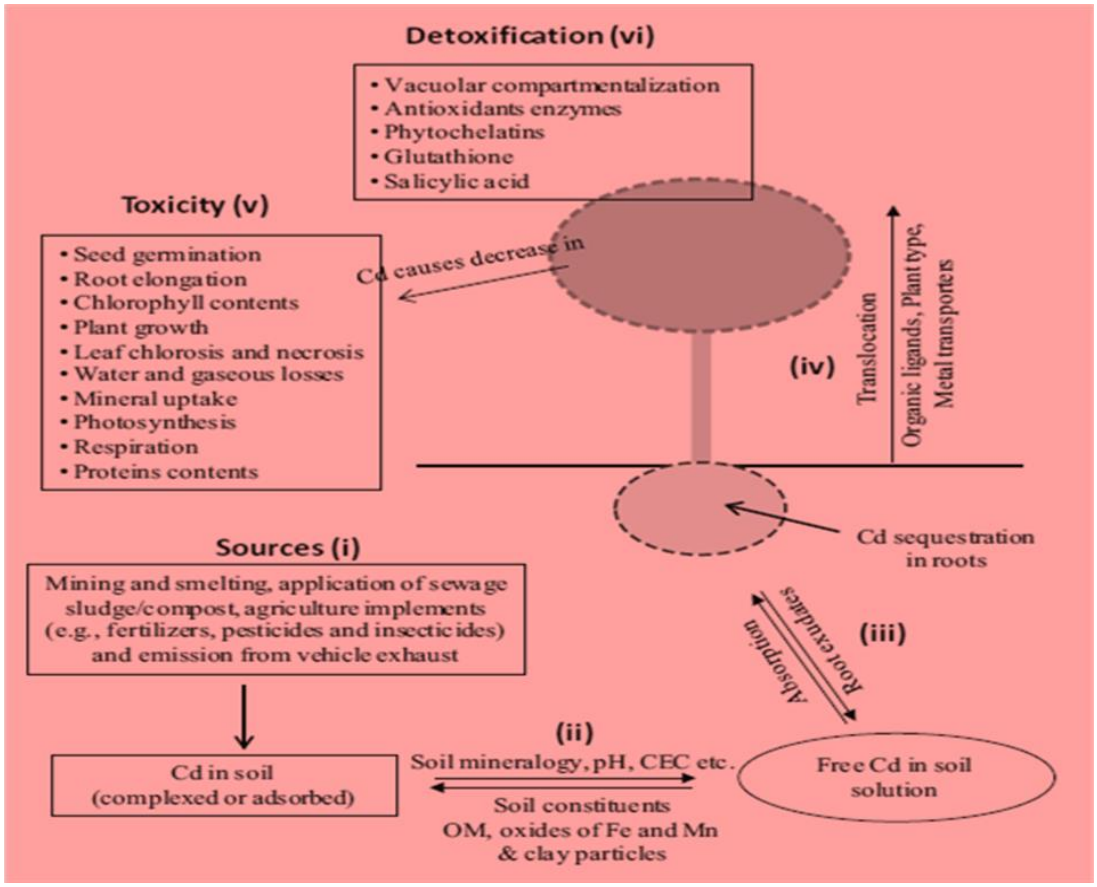


Figure 2.3: Biogeochemical behaviour of Cd in soil-plant system. (Shahid et al., 2016)

In plants, a greater concentration of cadmium is accumulated in the tissues of the roots even when absorbed through the foliar systems. With soil pH being the main factor of the uptake of cadmium, the greatest absorption of cadmium is in the range of pH 4.5 to 5.5. In addition to soil pH, cadmium phytoavailability is also affected by soil carbonates. Cadmium solubility is greatly influence by soil pH and also organic matter. Above pH 7.5, cadmium is not easily mobile (Kabata et al., 2001). The predominant forms of cadmium in soil solution at low pH are Cd^{2+}, $CdSO_4$ and $CdCl^{+}$ and the predominant forms in high pH are $CdHCO^{3+}$, $CdCO_3$ and $CdSO_4$ and the forms that exist at high alkaline pH are less bioavailable and the higher the pH the more they are adsorbed to soil particles and thereby reduction in plant uptake. 99% of cadmium is bound to colloidal portion of soil such as clay and humus particles. The bioavailability, speciation and partitioning is mostly control by the soil pH and at different soil pH, cadmium exist in different chemical forms. Soil pH and Cd bioavailability has been explored greatly and in phytoremediation of soil

cadmium contamination, the pH of the soil is lowered to enhance the uptake (Shahid et al., 2016).

The formation of complexes between soil organic matter and cadmium makes SOM to play a vital role in Cd bioavailability. It has been reported that humic substances bind Cd2+ stronger than the major inorganic ligand at high pH. The effect of humic substances to the phytoavailability of metals depends on the concentration, source, form of Cd in soil and physicochemical quality. Soils that have higher organic matter reduce the uptake of Cd by plants effectively and also remove Cd from soil solution due to the Cd-sorption on to the functional groups of humic substances. By the alteration of pH, cation exchange capacity, porosity and particle size distribution, SOM can affect the bioavailability of cadmium. The transport of cadmium from roots to the shoots occurs through the transpiration-driven xylem loading. A study carried out by Zhao et al. (2006) show that a decrease in transpiration led to the reduction of Cd in the aerial tissues (Shahid et al., 2016).

Cadmium being the most ecotoxic metal that causes adverse effects in plant metabolism and biological activities in soil is of increasing environmental concern. Cadmium is phytotoxic and its capacity to cause toxicity is related to the inhibition or destabilisation of enzyme activities. For example; anthocyanin and chlorophyll pigments inhibition in plants. Cadmium has a high affinity for sulfhydryl groups and complexes with metallothionein-like proteins and this is an important characteristic of cadmium. Due to the affinity of Cd to sulfhydryl, it is likely to be in high concentration in the protein sites of plants (Kabata et al., 2001). The accumulation of cadmium in plants affects the morphology and growth of plants adversely and above the toxic threshold, the biochemical and physiological functions are negatively affected. Above the Cd concentration of 5-10 mg/g leaf dry weight, plant death may occur. Very high concentration of Cd at the cellular level can cause cell cycles and cell division changes, chromosomal aberrations, and reactive oxygen species production. Excess reactive oxygen species production causes cell death as a result of oxidation of protein, damage of DNA and RNA, lipid peroxidation, and inhibition of enzyme (Shahid et al., 2016).

The interaction of cadmium and other heavy metals can either have synergistic effects or antagonistic effects on the plants. Cd-Fe interactions have a relation to the disturbance of the photosynthetic apparatus. Cd-Cu interaction has a complex nature and Cu inhibits the absorption of Cd. General effects of elevated cadmium in plants are; root damage and retardation of growth, inhibition of photosynthesis, chlorosis, CO2 and transpiration disturbance and destruction of cell membrane permeability. In nutrient medium, cadmium concentration of 50 to 75 μM/L, greatly cause reduction of chloroplast photochemical activities (Kabata et al., 2001). The symptoms of phytotoxicity induced by cadmium are stunted growth, root elongation, chlorosis, inhibition of photosynthesis, lipid peroxidation, and impaired seedling

development. Toxicity at cellular level include increased generation of ROS, deterioration of lipids, nucleic acid and proteins, cell redox interruption, and DNA strands cleavage. The phytotoxic effect of cadmium is linked to ATPase activity disruption, photosynthesis reduction, disruption of nutrient and water uptake and transport, reduction in respiration and growth of plant, nitrogen metabolism alteration, chlorosis, inhibition of photosynthesis, and reduced plant length (Shahid et al., 2016).

Table 2.3: Threshold values of Cd in edible plant parts established by the Codex Alimentarius Commission of FAO/WHO (CODEX 2006)

Food	Threshold values (mg/kg)	Remarks
Cereals, pulses and legumes	0.1[a]	Excluding bran and germ, wheat grain, rice, soybeans and peanuts
Wheat grains and rice	0.2[b]	Including bran and germ
Soybeans and peanuts	0.2[b]	
Vegetable, including potatoes (edible part)	0.5[b]	Excluding leafy vegetables, fresh herbs, stem and root vegetables, fungi, tomatoes and peeled potatoes
Peeled potatoes, stem and root vegetables	0.1[b]	Excluding celeriac
Leafy vegetables, fresh herbs, celeriac and fungi	0.2[b]	

[a]Indicates guideline level; [b]Indicates maximum level (Shahid et al., 2016)

2.3 Chromium

Chromium is relatively found in trace amount in soil and the most common is the trivalent form (Cr (III)). In plants Cr is a nonessential element (Ding et al., 2014). Chromium form both anionic and cationic complexes and have variable oxidation states. Naturally, chromium has two valence states; +3 (chromic) and +6 (chromate). The chromate ions are very mobile and can be absorbed by clays easily. The state of chromium in soil and its transfer from soil to plant is governed by adsorption and reduction (Kabata et al., 2001). The sources of Cr pollution in the environment can

be from volcanic activity, natural, geogenic or anthropogenic sources, see figure 2.4 (Shahid et al., 2017). The Agency for Toxic Substances and Disease Registry ranked Cr 7th out of 20 hazardous chemicals. Soluble chromate is toxic to plants and animals.

Chromium is of high concern in the environment due to its variable oxidation state. Cr has no metabolic function in plants and not required by plants and is phytotoxic. Cr(III) occurs as cation while Cr(VI) occurs as oxyanions (examples are dichromate, hydrochromate and chromate). The hexavalent Cr is very mobile in soil and more stable. The toxicity of Cr (VI) is greater than that of trivalent Cr and has been observed in soil at <1 mg/kg Cr (VI). The less mobility of trivalent Cr is its ability to precipitate at natural pH. The oxidation states of Cr ranges from -2 to +6 but the most stable chemical forms are Cr(III) and Cr(VI). The both forms are different in terms of toxicity, bioavailability, absorption and translocation. Many studies carried out have reported different natural and background levels of Cr but the natural levels found in the earth's crust ranges from 0.1 to 0.3 mg/kg. The different studies showed that majority of the soils have chromium levels of 15 to 100 ug/g and the level increases as the clay content increases. An estimation of 64 mg/kg Cr accepted level in soil for environmental health protection. The maximum allowable level of total Cr in agricultural soil varies from country to country, see table 1.1(Shahid et al., 2017).

One of the causes of Cr contamination is organic fertilizers such as phosphorus fertilizers which contain high quantity of Cr. Tannery sludge added to soil which contain up to 2.8% chromium is the most hazardous anthropogenic source (Kabata et al., 2001). Though most soils have high amount of chromium, the availability to plants is limited and the content of chromium in plants is mainly controlled by the soluble chromium in soils and some other soil and plant factors. The distribution of chromium in a plant is not uniform, roots have the highest concentration follow by leaves and stem and the lowest is in grains. The concentration of chromium also varies among vegetables with the highest concentration found in the root of the Brassicaceae family. The lowest concentration of chromium was found in the roots of *Allium sp* (Kabata et al., 2001). Plants take up both Cr (VI) and Cr (III) but the mechanism of uptake is not fully understood. Cr is a non-essential metal in plants and has no metabolic function and no specific uptake pathways have been reported. It has been suggested that Cr uptake is through specific essential ions carriers in plants and the uptake depends on the type of plants and species of Cr (Shahid et al., 2017).

The uptake mechanism of Cr (III) is passive whereas that of Cr (VI) is an active process requiring energy. Cr^{3+} is not translocated through cell membrane as a result of its low solubility and its binding to cell walls of roots (Kabata et al., 2001). The structural similarity of Cr (VI) to both phosphate and sulfate shows that its uptake is by phosphate or sulfate transporter. The soil-plant transfer index of Cr (VI) is higher

than that of Cr (III) because of its high solubility and adsorption. The bioavailability and mobility of Cr in soil is greatly controlled by clay contents, pH, CEC, and organic carbon. These physicochemical properties are used to explain the phytotoxicity of metals (Ding et al., 2014).

The transfer of Cr from soil to plants is affected by two major types of factors: plant physiology such as root surface area, type of plant, transpiration and type of root secretions; and properties of soil such as pH, CEC and texture. The pH of soil is an important parameter that governs the adsorption/desorption and speciation of Cr in soils. The bioavailability, mobility, and sorption/desorption is controlled by soil organic matter due to its ability to convert Cr (VI) to Cr (III). The reduction of Cr (VI) to Cr (III) by SOM is depended on pH, redox potential and CEC. Higher SOM create a condition for reduction. Increase soil CEC leads to increased sorption of cationic Cr (III) by SOM (Shahid et al., 2017). The solubility of Cr(III) at pH<5.5 is low and it precipitates above this pH making its compound stable in soil whereas Cr(VI) is very unstable and in both alkaline and acidic soil it is mobilized. Due to the influence of soil pH on Cr bioavailability, safe levels at various pH have been suggested such as 150 mg/kg at pH<6.5; 200 mg/kg at pH 6.5-7.5; and 250 mg/kg at pH>7.5 (State Environmental Protection Administration of China). The elevated concentration of chromium in plants is due to the anthropogenic activities such as some phosphate fertilizers which contain up to 600 ppm of chromium in soil. Chromium is a known plant toxic metal that is detrimental to their growth, and also affects the physiological and biochemical processes.

The toxicity of Cr is observed in different levels from low yield to growth abnormality of roots and leaf, mutagenesis and enzyme inhibition. The effects of excessive level of chromium in tissues of plants are physiological, biochemical and morphological. The toxicity can be broken down as reduced plant growth, alteration of enzymatic activities, modification of chloroplast and cell membrane, damaged root cells, chlorosis and reduced pigment content. Chromium inhibits seed germination by decreasing the availability of sugar and the action of amylase enzyme in the young embryo. Additional toxicities of Cr to plants are root growth retardation which has been seen in a study with *Phaseolus vulgaris* (0.5mM Cr VI). The decreased length of root is attributed to decreased division of root cells. Higher levels of Cr in plants are responsible for the generation of ROS which may lead to cell death because of DNA and RNA mutilation, protein oxidation, enzyme inhibition, lipid peroxidation and chromosomal aberration (Shahid et al., 2017). Chromium is known to influence photosynthesis negatively by inducing the production of ROS. Chromium also inhibits photosynthesis by alteration of ultrastructure in the chloroplast, decreases chlorophyll a, chlorophyll b and also carotenoids. The entire process of photosynthesis is affected by chromium stress; enzyme activities, fixation of carbon dioxide, electron transport and photophosphorylation are affected. Ultrastructural changes in the chloroplast have

been observed *Hibiscus esculentus, Phaseolus vulgaris, Ocimum tenuiflorum*. The toxicity of chromium can be seen as chlorosis in young leaves and on cereals, root injury, wilting of tops and brownish red leaves (Kabata et al., 2001).

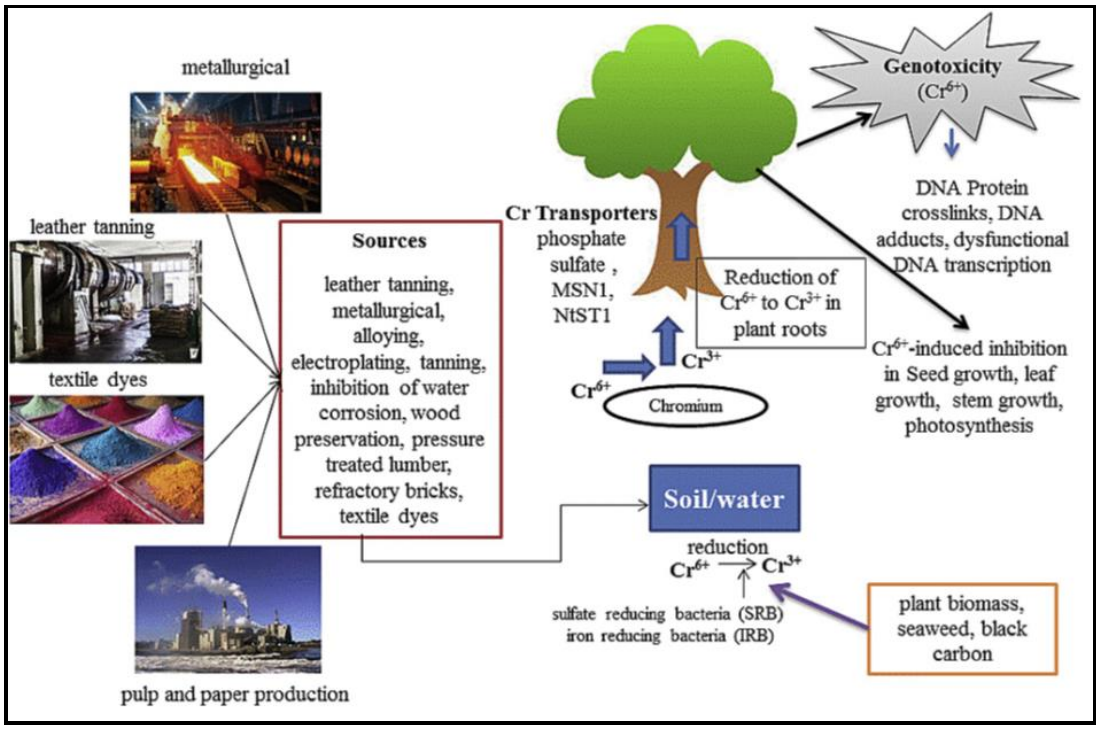


Figure 2.4: The biogeochemical behaviour of Cr in soil-plant system and its effect (Shahid et al., 2017)

2.4 Manganese

Manganese is the 12th most abundant element in the earth's crust with an atomic number of 25. Manganese is found in sufficient amount in the soil and is also being enriched by anthropogenic activities which are a threat to plants and animals (Anjum et al., 2015). Manganese has variable oxidation states such as 0, +2, +3, +4, +6 and +7. In biological systems, only the +2, +3, and +4 states occur with +2 being the most soluble form in soil and therefore available in plants. Mn ranges in the lithosphere is between 350-2000 ppm and forms minerals with other elements. It occurs as Mn2+, Mn3+, and Mn4+ but Mn2+ ion is the most frequent and replaces divalent ions such as Fe2+ and Mg2+ in silicates and oxides (Kabata et al., 2001). The manganese level in soil is in a range of 450-4000 mg/kg soil and the natural level in soil is in the range of 1.0-4000 mg/kg d.w (Anjum et al., 2015).

There are three forms of manganese in the soil; soluble Mn2+ which is phytoavailable and insoluble Mn3+ and Mn4+ which are easily reducible. Manganese is a trace element with some physiological functions in plants such as photosynthesis, redox processes, serves as enzyme co-factor in PSII (Fernando et al., 2015). The soluble form of Mn in soil is easily taken up by plants, thus the proportion of soluble Mn in plants is directly related to that in soils. The relationship of Mn concentration in plants and the soil pH is indirectly proportional; an increase in soil pH negatively affects plant Mn concentration. But soil organic matter has a direct and positive relationship with plant Mn concentration. Excess concentration of phytoavailable form of Mn is related to factors such as High limed soil (pH of up to 8); acid soils of pH 5.5 or less and anaerobic and poorly aerated soils due to flood or waterlog or compact soils. The uptake and translocation of Mn in plants is known to be rapid as it does not bind to ligands and root tissues or to xylem fluid. Mn is transported as Mn2+ ions and the phloem exudate has a lesser Mn concentration than leaf tissues; this lower concentration of Mn in the phloem vessel is responsible for the lower concentration of Mn in seeds, fruits and storage roots (Kabata et al., 2001).

The frequent reactions of Mn in soil are hydrolysis and redox reactions as the solubility is mainly dependent on pH and redox potential. The mobility of Mn is controlled by two factors; reduction of MnO2 and formation of complex by root exudates in the soil around the plant roots. The Mn in the topsoil is mostly bound to fulvic acid but the Mn2+ ion is highly ionized (Kabata et al., 2001). The solubility and bioavailability of manganese is highly control by soil pH. Higher pH favours adsorption of manganese into soil particles which cause decrease in manganese availability. Manganese +2 is absorbed by epidermal cells of roots by active diffusion (Anjum et al., 2015). The bioavailability of Mn is affected by the soil Mn content, CEC, and pH. The uptake of manganese occurs in two stages: (i) uptake of Mn+2 in the apoplast of the root cells; where negatively charged cell wall constituents adsorb Mn+2. The adsorption is rapid, irreversible and nonmetabolic. (ii) Mn2+ is taken up by symplast in a slow and nonmetabolic process. Manganese distribution is unequal in plant systems; aerial tissues accumulate more Mn than the roots. Mn is transported through the xylem with a high mobility from the roots to the shoots and leaves by aid of the transpiration stream. Mn is relatively immobile in the phloem transport system. The distribution of Mn2+ at cellular level is unequal; highest in the vacuoles followed by chloroplast, cell wall and endoplasmic reticulum (Anjum et al., 2015). On acid soils, the toxicity of Mn is a high threat to the vegetation as soil acidity below pH 5.3 negatively affects plants. Oxides of Mn are solubilised by acidic and hypoxic soils to soluble Mn2+ which is known to induce plant toxicity. Manganese toxicity is very common in Puerto Rico, Eastern Australia, Brazil, Hawai and tropical Africa due to climate effects and natural processes (Fernando et al., 2015).

Manganese is an essential element in plants but high concentrations of Mn is toxic to plants and can lead to inhibition of many processes. Elevated Mn concentration causes Mn phytotoxicity which is mediated through the inhibition of glutathione reductase and ascorbate peroxidase which are important free radical mitigating antioxidative enzymes. High level of Mn in plants also causes oxidative stress through the antagonism of metals of similar structures thereby causing deficiency in enzyme cofactors responsible for antioxidative activities (Fernando et al., 2015). Elevated levels of Mn has resulted in chromosomal and mitotic alterations, disrupted cell homeostasis, generation of reactive oxygen species and altered metabolic processes (Anjum et al., 2015).

However, high concentrations of Mn in the cells cause production of ROS, and antagonism of similar ions. The manifestation of elevated Mn is seen as chlorosis, crinkling and dark inclusions. Excess Mn lead to chlorosis, decreased rate of photosynthesis, reduction in the size of chloroplast, leaf necrosis, inhibit synthesis of chlorophyll. The toxicity of Mn targets mainly the photosystem I. Manganese toxicity also leads to cell disintegration, endoplasmic reticulum, mitochondria and Golgi apparatus structural changes (Anjum et al., 2015). Excess Mn concentration in soil makes the Mn^{2+} ions to compete with Mg, Ca, K, Fe thereby disrupting their uptake and nutrition. Antagonism of Fe by Mn is widely known to occur in acidic soils. Fe and Mn generally have interrelated metabolic functions. The normal Fe:Mn ratio for a healthy plant is 1.5:2.5 (Kabata et al., 2001).

2.5 Lead

Lead is a non-biodegradable heavy metal that is of greater threat to the population. Lead has an atomic number of 82 and atomic weight of 207.19. The melting point of Pb is 327.5 o C and boiling point 1740 o C (Tangahu et al., 2011). Lead has a relative abundance in the earth's crust of approximately 15 ppm. The natural Pb level in plants that grow on uncontaminated soils range from 0.1 to 10 ppm (DW). Reports has shown that more than 100 ppm of Pb has been found in Britain, Japan, Ireland and Denmark and this higher level is indicative of pollution (Kabata et al., 2001). There are several anthropogenic sources of Pb pollution of soils that ranges from industrial sites, leaded fuels, orchard sites where Lead arsenate was used and old lead pipes. The accumulation of Pb in soil is mostly in the upper 8 inches portion of topsoil and it is very immobile with long term contamination. The high lead levels in soil cannot return to normal level without the remedial actions because it cannot undergo biodegradation. When the soil is polluted with Pb, the exposure and effect is long term due to the non-biodegradable characteristics of the metal (Tangahu et al., 2011).

Environmental contamination of Pb has detrimental effects on the productivity of plants and the health of humans. Due to fast industrialisation, Pb has become the major common environmental pollutant according to EPA. Pb is not an essential metal in plants but due to its presence in soil by anthropogenic sources such as Pb fertilizers and automotive exhaust, it is taken up by plants (Lamhamdi et al., 2011). It has been reported that the highest Pb concentrations are found in rich top organic uncultivated soils. Organic matter is an important reservoir of Pb in contaminated soils. The uptake, translocation and toxicity of Pb2+ vary with the plant species and tissues. It was found that *Mimosa caesalpiniaefolia* has more tolerance to high concentrations of Pb2+ than *Erythinna speciose* in soil. Research has shown that some dicotyledons have very high accumulative capacity for Pb2+ than some monocotyledons (Shen et al., 2016). Due to the insolubility resulting from the precipitation of Pb in soil, Pb contamination was of less concern. However, the concentration of Pb in plant roots is correlated to that in the soil and this is an indication of Pb uptake by plants. Factors that enhance the uptake and translocation of Pb by plants are low soil pH, organic ligands and low soil phosphorus content (Kabata et al., 2001).

The uptake of Pb by plant roots is passive and the rate of uptake can be reduced by low temperature and liming of soil. The absorption of Pb is by root hairs and mostly stored in the cell wall. The uptake of soluble Pb in solutions by plant roots is greater and the rate increases as the concentration of soluble Pb in solution increases with time. However, the translocation of Pb from roots to shoots is very slow and limited as only 3% of the Pb concentration in the roots is been translocated to the shoots. Therefore, higher amount of Pb is accumulated in the roots of plants. Liming has a negative impact on the solubility of Pb and therefore soils with higher pH content decrease the solubility of Pb and precipitate Pb as phosphate, hydroxide or carbonate. These complexes are stable. Pb solubility increase with increasing acidity and therefore plants growing on acid rich soils tend to have higher levels of Pb (Kabata et al., 2001).

Pb is toxic to plants, microorganisms and animals. The life of a plant begins from seed germination which is a complex process that involves enzymatic reactions. Pb is known to inhibit seed germination (Lamhamdi et al., 2011). The toxicity of Lead is dependent on soil properties such as SOM, CEC, and pH (Cheyn2012). Pb toxicity depends on the soil properties, Pb concentration, type of salt and plant species. Excess Pb concentration affects functional groups in macromolecule, enzyme activities, and thus plant water status, photosynthesis and mineral nutrition are affected. Toxic levels affect major processes such as seed germination, dry ass of shoots and roots, and seedling growth (Lamhamdi et al., 2011).

Lead induces oxidative stress in plant parts as a result of ROS production. Due to the oxidative stress produce by Pb, cell damages occur which lead to reduction of plant

productivity. Lead toxicity includes inhibition of chlorophyll production, plant growth, root elongation, transpiration, seed germination, seedling development and cell division (Kumar et al., 2013). Pb toxicity causes adverse effects on seed germination, root elongation, plant growth, antioxidant enzymes system, seedling development, chlorophyll production (Shen et al., 2016). Pb is a phytotoxic metal that causes inhibition of ATP production, alter cell membrane permeability, that is, Pb reacts with functional groups of enzymes that are involve in metabolism; reacts with phosphate groups of ADP and ATP; and also replaces essential ions, Pb also causes production of ROS which is responsible for lipid peroxidation and DNA damage. There exist antagonism between Pb and Zn and this negatively affects their translocation from plant roots to shoots (Kabata et al., 2001).

2.6 Nickel

Nickel is a heavy metal with atomic number 28 and is the 22nd most abundant element in the earth's crust. Ni exists in two forms either in combination with iron or as a free metal in igneous rocks. The natural level of nickel in agricultural soil is in the range of 3.0 to 1000 mg/kg but contaminated soils have a range of 200 to 26000 mg/kg. The distribution of Ni in the earth's crust is similar to that of cobalt and iron, even though weathering facilitates its mobilization. Ni can migrate over long distances and is relatively stable in aqueous solutions. Ni as a metal has valence states that range from +1 to +4 and the +2 valence state of nickel is present in the environment more than the others. Divalent nickel is more available to plants (Anjum et al., 2015). The Ni content of vegetables ranges from 0.2 to 3.7 ppm (DW) and in other plants such as covers, grasses, and wheat grains ranges from 0.1 to 2.7 ppm (DW) (Kabata et al., 2001).

Anthropogenic activities have increase soil content of Ni massively and some of the sources of pollution are metal processing, combustion of coal and oil, sludge, phosphate fertilizers. The industrial sources have significantly increases the concentration of Ni in soils and make Ni a serious pollutant. The organic chelated form of Ni in sewage sludge is readily available to plants and thus making it highly phytotoxic (Kabata et al., 2001). Many anthropogenic activities release high level of Ni to soil. More than 60% of anthropogenic source of nickel enters into the soil and is responsible for majority of the pollution of soil by nickel.

The uptake of Ni from soil is mostly by plant roots through passive transport though can also be taken up by active transport. The uptake of soluble Ni is also facilitated by cation transport system. Active and passive transport mechanism of Ni is changes with the soil pH, Ni concentration in soil, plant species, the presence of other metals and oxidation state. The uptake of Ni^{2+} is in two stages; a rapid stage that is followed by a slow linear phase (Anjum et al., 2015). The uptake of Ni is affected

both by plant factors and pedological factors with soil pH being the dominant factor. Berrow and Burridge found that the Ni content of oat grains was decreased by a factor of 8 when the soil pH was increased from 4.5 to 6.5 showing that the soil pH and Ni uptake is indirectly proportional. The uptake of Ni varies with plant species, some plants are hyperaccumulator such as *Alyssum sp*, berries and grains (Kabata et al., 2001). The bioavailability of Ni to plants is governed by Fe oxides/hydroxides, CEC, soil pH and SOM. The translocation of Ni from roots to other parts of the plant is very rapid due to the high mobility of Ni in plant systems. Ni can be easily translocated from older leaves to younger ones due to its high mobility. The movement of Ni within the plant is controlled by transporter proteins, organic acids and metal-ligand complexes and the flow of xylem sap aids in the rapid translocation of Ni from roots to shoots (Anjum et al., 2015). The content of Ni is highest in clay and loamy soils. In the U.S, soil Ni ranges from 5 ppm to 150 ppm and throughout the other parts of the world, the range of soil Ni is 0.2-450 ppm. According to Kabata P. and Pendias H. (2001), the bonding of Ni to organic ligands is not strong but organic matter is capable of mobilizing Ni from oxides and carbonates. Soils that have high complexation ability such as polluted and organic rich soils support the mobilization of Ni. The solubility of Ni in soil and soil pH is inversely related, that is, lower soil pH favors Ni solubility and higher soil pH leads to lower solubility of soil Ni. Ni transport and storage is controlled metabolically and accumulation of this metal is both in leaves and seeds. The plant roots readily take up soluble Ni and the uptake of Ni by plants is directly proportional to the concentration of Ni in solution (Kabata et al., 2001).

Elevated level of Ni causes physiological and morphological changes in plant and also inhibits plant growth, productivity and development (Anjum et al., 2015). Although the phytotoxic mechanism of Ni is not well understood, there are some abnormal observations in plants that resulted from excess Ni over a long period of time. Some of the common symptoms of Ni toxicity in plants are restriction in plant growth, chlorosis, and plant injuries such as retardation in root development, nutrient absorption, and metabolism. There is also inhibition of photosynthesis and transpiration in acute Ni phytotoxicity (Kabata et al., 2001).

Elevated level of Ni has a negative effect on the physiological mechanism of plants and also on the growth of plants. The toxic effects of Ni in plants are as follows: irregular shape of flower, inhibition of germination process, distortion of plant parts, decrease growth of roots and shoots, reduction in the yield of crops, chlorosis, and reduction in leaf area. The presence of certain metal ions such as Cu^{2+}, Zn^{2+}, and Fe^{2+} are shown to inhibit the absorption and translocation of Ni^{2+} from roots to shoots by soy bean plant. The interaction of Ni and other trace metals have both antagonistic and synergistic effects (Kabata et al., 2001). Some of the metabolic and physiological effects of high Ni level in plants include: synthesis of chlorophyll, photosynthesis, plant water relations, absorption of mineral by roots, transpiration,

enzyme activities, nitrogen metabolism, and carbohydrate metabolism (Anjum et al., 2015). Oats which is a Ni sensitive crop contains 24 to 308 ppm (DW) Ni in the leaves when exposed to the metal. The toxic concentration of Ni in plants range widely among different plant species and reports have shown to be in the range of 40 to 246 ppm (DW). Excess Ni induces hydrogen peroxide accumulation and also causes oxidative stress by the generation of reactive oxygen species. The ROS can directly or indirectly interfere with the DNA repair system by causing point mutations. Reduction in the content of DNA and RNA in *Nigella sativa* and *T. aestivum* was seen when exposed to 10-25 ppm of Ni. In *Jatropha curcas*, DNA polymorphism was also observed which causes alterations in the sequence of DNA when it was exposed to excess Ni (Anjum et al., 2015).

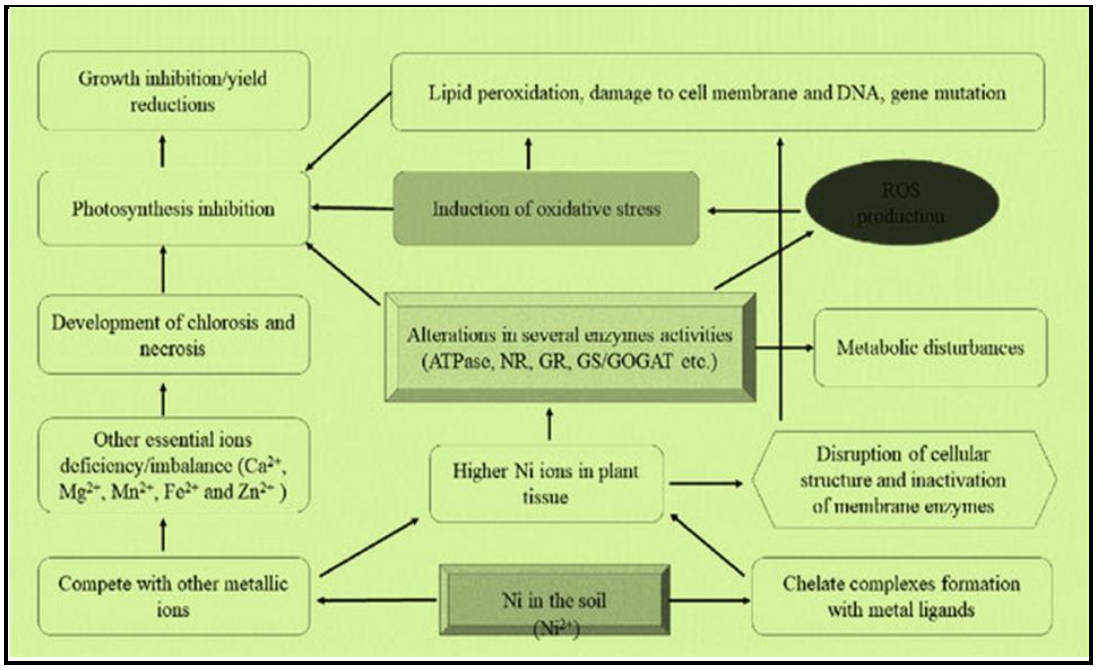


Figure 2.5: Possible mechanisms facilitating toxic effects of excessive Ni in plants (Shahzad et al., 2018)

3. REMEDIATION TECHNIQUES FOR HEAVY METAL CONTAMINATED SOIL

There has been increasing concern over the years on heavy metal contamination of soil around the globe and also many remediation techniques have been developed (Khan et al., 2000). Due to the non-degradable nature of heavy metals, it is of great importance to develop techniques other than degradation to reduce or eliminate their effects in soil and plants. Reports have shown that greater than 50 % of the contaminated sites worldwide are contaminated with heavy metals and most of them are found in developed countries such as Germany, China, U.S.A, Sweden and Australia due to their high level of industrialization (Khalid et al., 2017). The awareness of the detriments of elevated concentration of heavy metals in agricultural soil worldwide has improved the development of clean-up techniques for heavy metal contamination.

Remediation of heavy metal contaminated soils can be done on-site or off-site but the off-site (excavation and disposal) remediation just remove the problem from one site and shift it to another site with dangers during the transportation of the soil to landfill disposal. Soil washing is an alternative to excavation (Tangahu et al., 2011). The remediation approach of contaminated soil is influenced by the form (physical and chemical) of the heavy metal contaminant in soil. Therefore, the contamination site must be assessed accurately, obtaining information about the physical characteristics of the site and the contamination type and level in the site (Evanko et al., 1997). USEPA, 2017 stated that these factors have to be considered when choosing a heavy metal clean-up technique; site geography, cost-effectiveness, time requirement, characteristics of contamination, public acceptability, financial budget, goal of remediation, and implementation readiness (Liu et al., 2018).

The remediation techniques can be grouped into 2 categories; *in situ* techniques which involves on site remediation without excavation, and *ex situ* techniques which involve excavation, that is removal of the polluted soil for treatment. Ex situ treatment can be on-site or off-site. In situ techniques are preferred to ex situ because they have lower cost and minimal impact on the environment. The techniques used for the remediation of heavy metal contaminated soil are generally grouped under chemical, biological or physical methods although they can be used in combination. Majority of these techniques are environmentally-not-friendly, expensive and time consuming (Khan et al., 2000, Khalid et al., 2017). These techniques are grouped in 5 approaches: immobilization, toxicity reduction, isolation, extraction and physical separation. These approaches can be used in combination (Evanko et al., 1997).

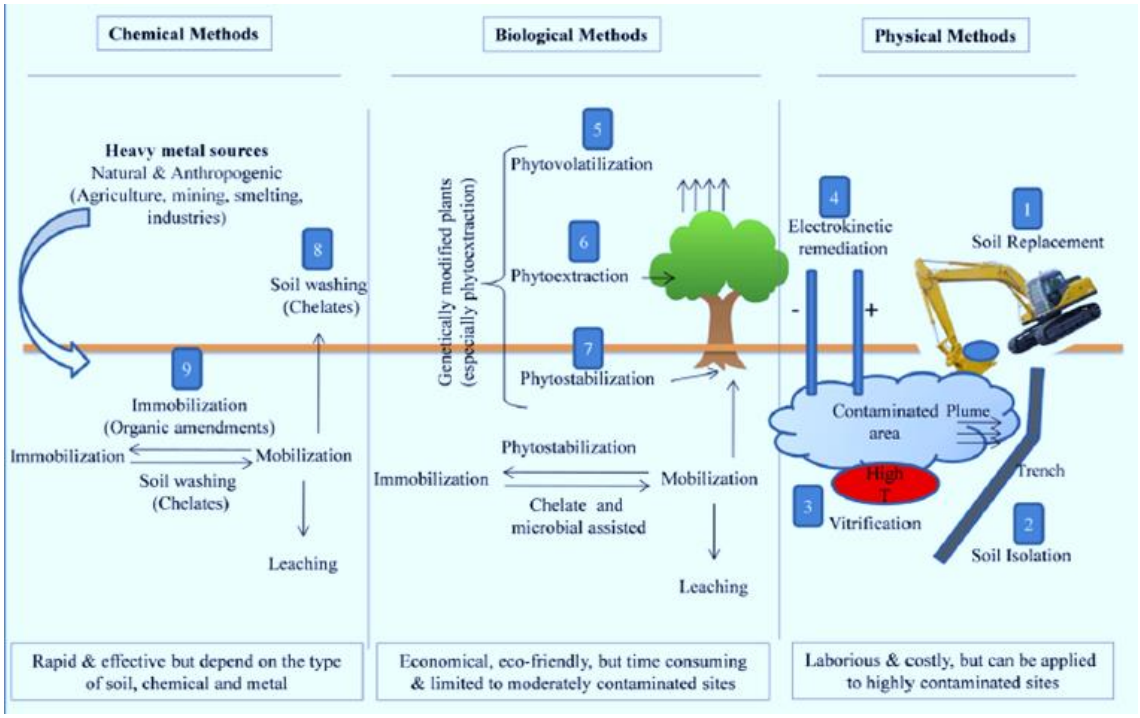


Figure 3.1: Categories of soil remediation methods (Khalid et al., 2017)

3.1 Physical remediation

3.1.1 Soil replacement

This is the process of replacing polluted soil with non-polluted soil either completely or partially and the excavated soil is disposed to landfills or treated to recover the metals. The frequently used techniques for soil remediation before 1984 were excavation, off-site disposal and soil replacement. Soil replacement boosts functionality of soil by mixing and reducing the concentration of heavy metals (Khalid et al., 2017).

3.1.2 Soil isolation

Soil isolation is the temporal separation of heavy metal contaminated soil from uncontaminated soil. This technique is used when other techniques are not physically or economically feasible to prevent further contamination. Isolation techniques are designed to set a containment area so that there is no movement of the metal contaminants or restrict the area of contamination from further

contaminating nearby fields. Contamination site can be isolated during site assessment and remediation to prevent movement. The contaminated area can be isolated temporarily to limit transport during the assessment and remediation of the site (Khalid et al., 2017, Evanko et al., 1997).

3.1.3 Vitrification

High heat can be applied to a contaminated soil that will lead to the reduction of heavy metal mobility with vitreous material (an oxide solid) as a by-product. This process is known as vitrification which is defined as the formation of vitreous material through the application of high temperature treatment in a heavy metal contamination site. Mercury for example is a heavy metal that can be volatilize by high heat and in the process of vitrification, the volatilized product must be collected for treatment or disposed as hazardous waste. Vitrification is a non-classical technique though it is very easy to apply and can be applied to wide range of heavy metal contaminated soil. Reports have shown that Zn, Cu, Mn, Ni, and Fe were immobilized at 1350 ^{o}C. Vitrification is achieved when electric current is applied to the contaminated soil by inserting arrays of electrodes vertically (Khalid et al., 2017, Evanko et al., 1997).

3.1.4 Electrokinetic remediation

Electrokinetic remediation is a new *ex situ* remediation technique and is cost effective. The principle of this technique is simple; in an electrolytic tank with saturated contaminated soil, an electric field gradient of acceptable intensity is applied on both sides and the heavy metals are separated by electrophoresis or electro-migration (Khalid et al., 2017, Liu et al., 2018).

3.2 Chemical remediation

3.2.1 Immobilization techniques

Immobilization is the process of limiting or reducing heavy metals bioavailability, mobility and bioaccessibility in soil by the addition of immobilizing agents (Khalid et al., 2017). Immobilization techniques don't really remove the contaminants but just reduce the mobility and bioavailability of the metal contaminants to plants. Basically, immobilization techniques accelerate precipitation, adsorption, and complexation reactions (Gonzalez et al., 2012). Heavy metal immobilization can be done by increasing the pH of soils by liming. The formation of insoluble hydroxides of Cd, Cu, Ni, and Zn greatly reduced their solubility in soil (Khan et al., 2000). The frequently used agents are cement, minerals, zeolites, clay, iron oxide, lime,

biosolids, CaO, biochar, medical stone, fly ash, microbes, and phosphate fertilizers. There are also low cost industrial residues that are being used as heavy metal immobilizing agents, they include red mud, termitaria, and industrial eggshell. Biochar is widely used among all the amendments and is an old technique that has its roots from slash-and-burn agriculture due to its capacity to limit heavy metals such as Zn, Cd and Pb mobility in soil (Ali et al., 2017).

There are many methods to immobilize metal contaminants using either chemical reagents and/or biological materials to bind the polluted soil. Immobilization methods include solidification/stabilization, liming and biochar, encapsulation, chemical redox and soil washing (Evanko et al., 1997).

3.2.1.1 Solidification/Stabilization

These are the most used remediation techniques for metal contaminated sites. The general approach for solidification and stabilization is the mixture of agents to the polluted soil. Stabilization which can also be termed fixation uses chemicals that react to make the contaminant less mobile while solidification involves processes that make the matrix solidifies altogether with the contaminant, that is the solidified matrix binds the contaminant physically (Evanko et al., 1997). Precipitation of the metals by hydroxides within the matrix is the superior mechanism for immobilizing heavy metals in contamination sites. There are two types of binders; inorganic which are blast furnace slag, fly ash and cement; and organic binders which are bitumen (form a crystalline, glassy framework around the contamination site). The limitation of these techniques are metal that exists as anions such as Cr(VI), arsenic; metals with high solubility hydroxides such as mercury cannot be cleaned using these techniques (Evanko et al., 1997).

3.2.1.2 Biochar and Liming

Biomaterials are also used as immobilizing agents in heavy metal contaminated soils due to their low cost and availability. Biochar, an example of biomaterials have been widely used to remediate heavy metal contaminated soils. Biochar which is also called biological charcoal is a porous, carbon rich charcoal produced by pyrolysis of organic residues (such as animal wastes, biosolids, crop residues, municipal wastes, and wood) at very high temperature. Biochar has been proven to be effective in enhancing heavy metal sorption, and reduction in phytoavailability and mobility (Khalid et al., 2017). Biochar can accept or donate electrons to metals (Omena et al., 2017). Biochar is an excellent sorbent of metal contaminants due to its large surface area. The various mechanisms of biochar use to immobilize metal contaminants are; carbonates, hydroxides or phosphate development; the d-electrons of metals are adsorbed by the p-electrons of biochar surface; precipitation due to the high biochar

pH (example is Pb immobilization); the cations interacts with the functional groups through electrostatic binding (example is arsenic). Biochar also immobilizes heavy metals by formation of precipitates, heavy metal absorption, electrostatic interaction ions exchange, and chelate formation (Ali et al., 2017). Biochar also cause adsorption of metal cations to soil particles indirectly by pH increase thereby immobilizing the metal contaminants (Brendova et al., 2016). Biochar can also be used to restrict the uptake of metals by crops thereby enhancing food safety (Li et al., 2018). Biochars increase the soil physicochemical properties such as CEC, pH, and nutrient contents of loamy soil and also limit the phytotoxicity and bioavailability of Pb, Ni, and Co (Mohamed et al., 2017). Zhai et al. (2018) reported that lime and biochar have been effective in immobilize heavy metals in contaminated soil and improve the biomass of plants (Zhai et al., 2018).

Biochar amendment boosts the biological and physico-chemical properties of soil which are essential for the fertility of soil and productivity of plants. Some properties of biochar that makes it more suitable for heavy metal remediation are large surface area, capacity to hold water and nutrient, resistant to decomposition in soil, alkaline pH, and high CEC. It has been reported that biochar can improve the defense mechanism of plants by improving the activities of the antioxidant enzymes of plants (Ali et al., 2017). The addition of amendments is not completely effective in immobilizing all metals; some might effectively immobilize one metal but same time increase the mobility of another metal while in some, the effectiveness of combined amendments is reduced than when applied individually. The effectiveness of this technique can be analysed by measuring the solubility and bioavailability or can analyse the lixiviates (Gonzales et al., 2012).

Studies of biochar and Brassica have been carried out to investigate the translocation of heavy metals in roots and shoots. Brassica has been used for phytoremediation due to its fast growing and hyperaccumulator capacity and is commonly grown around oil production area. The combination of biochar and lime as a treatment technique also improved soil environment and enhanced the soil microbial community (Zhai et al., 2018).

3.2.1.3 Encapsulation

Encapsulation is the process of mixing products such as lime, asphalt or cement with heavy metal contaminated soil to form solid blocks that prevent contamination of nearby soils and thus immobilize the heavy metals. Cement is the binding material of choice due to its cost-effectiveness, availability and versatility (Khalid et al., 2017).

There are other immobilizing agents such as alginate, chitosan, agar, polyvinyl alcohol, and polyacrylamide that can be used in encapsulation. In oil and heavy

metal contaminated soil, the combination of concrete and lime has been shown to be very effective (Khalid et al., 2017).

3.2.1.4 Chemical Redox and Neutralisation

There are chemical reactions that can transform the toxic metal to relatively non-toxic and decrease the mobility of metals. Chemical treatment can be done ex situ or in situ. These are oxidation, reduction and neutralization reactions (Evanko et al., 1997).

Chemical oxidation functions by alteration of the oxidation state of the metal atom via electrons loss. There are commercial chemical oxidizing agents available for treatment such as chlorine gas, potassium permanganate, hypochlorite and hydrogen peroxide (Evanko et al., 1997).

Chemical reduction functions by the addition of electrons to the metals to change their state of oxidation. The commercial reducing agents are ferrous sulfate, sulfite salts, sulfur dioxide and alkali earth metals such as Na and K. The process of metal oxidation and reduction leads to the detoxification, precipitation or solubilisation of the metal contaminants.

Chemical neutralization treatment aims at balancing the pH of soils with high acidity or alkalinity. Neutralisation can be used as a pre-treatment before chemical oxidation or reduction and is also use to precipitate insoluble metal salts (Evanko et al., 1997).

The set back of chemical treatment is their non-specific nature which can trigger the reaction of other reactive metals and this might lead to the mobility of the metal or making it more toxic. Chemical treatment as a whole can be used as pre-treatment method to prepare the contaminated site for other techniques such as solidification/stabilization techniques. Chromium contaminated soil can be remediated by chemical reduction where Cr(VI) is reduced to Cr(III) and the Cr(III) can easily be precipitated over a wide range of pH by hydroxide. Arsenic contamination on the other hand can be treated by either stabilization or chemical oxidation. The stabilization of arsenic is done by precipitation and coprecipitation with Fe(III) while arsenite is oxidized to arsenate which is less soluble, less mobile and less toxic as compare to arsenite (Evanko et al., 1997).

3.2.2 Soil washing

This is an *ex situ* method that involves the removal of heavy metal from contaminated soil by the used of extractants. In the process of soil washing, the excavated soil is mixed with suitable extractant solution. The selection of the extractant solution depends on the type of soil and metal. The mixture is thoroughly mixed for a given time period, and the metals moved from soil to a liquid layer

through chelation, precipitation, adsorption or ion exchange which are being separated. Example of extractant agents are $FeCl_3$, surfactants, organic acids, cyclodextrins and synthetic chelating agents such as EDTA and Ethylenediamine-N,N'-disuccinic acid (EDDS) (Khalid et al., 2017, Marques et al., 2009). Soil washing can be combined with other immobilisation techniques for a better soil remediation as shown in figure 3.2.

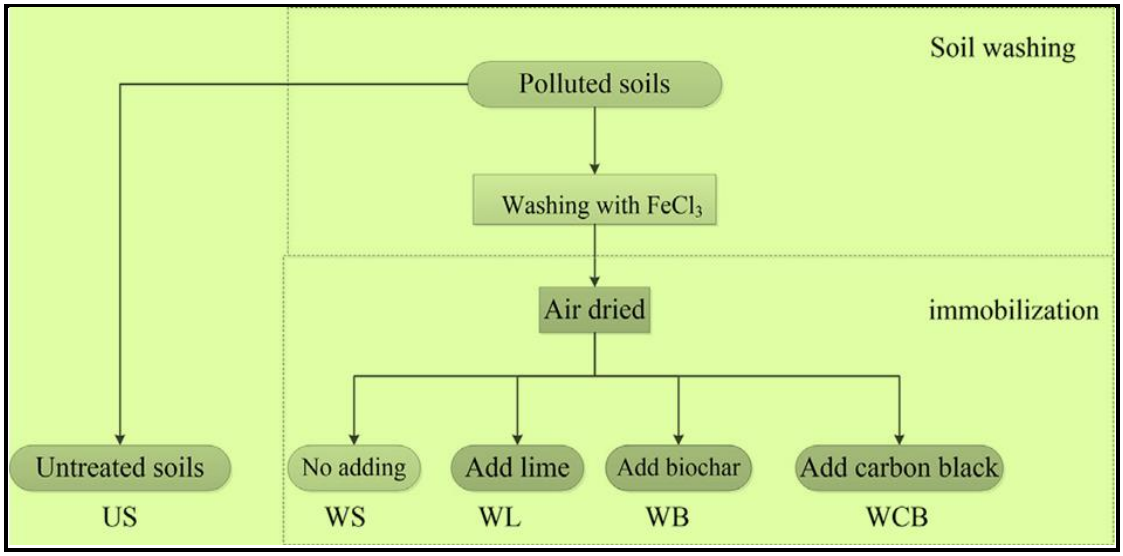


Figure 3.2: Combination of soil washing and in situ immobilization (Zhai et al., 2018)

3.3 Biological remediation

Bioremediation, also known as biological remediation, is a technique that uses biological systems such as plants and microbes to remediate heavy metal polluted soil. The advantage of this over conventional techniques is its non-invasiveness, cost-effectiveness and ability to give permanent solution and also re-establishing the natural soil condition (Khalid et al., 2017). Metal contaminated soil can be treated biologically. Biological treatment techniques utilizes the ability of plants and microbes to perform clean-up of metal contaminated site by their natural processes (Evanko et al., 1997).

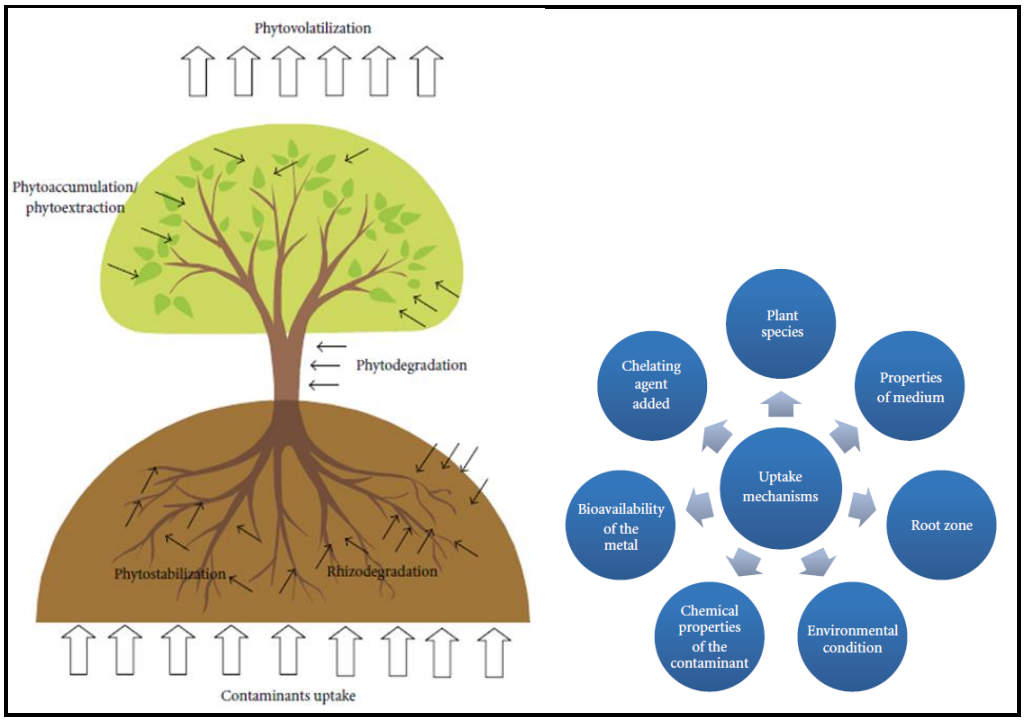
3.3.1 Bioaccumulation

Bioaccumulation is the process whereby living organisms or inactive biomass take up metals from contaminated soil. Plants and microbes have the capability to accumulate heavy metals in their tissues by natural processes. The accumulation occurs by ion exchange and complex reactions at the cell wall, and also extracellular

and intracellular precipitation. Inactive biomass takes up metals by ionic group adsorption at the cell surface (Evanko et al., 1997).

3.3.2 Phytoremediation

Phytoremediation is a clean-up process where plants are used to remediate metal contaminated soil (Evanko et al., 1997). Phytoremediation can also be called agro-remediation, green remediation, botanoremediation or vegetative remediation and can use either natural plants or genetically modified plants for heavy metal clean-up in soil (Mohamed et al., 2017). This is an effective, environmental friendly and affordable remediation technique as compare to the chemical techniques. Hyperaccumulators are plants that have high metal-accumulating capacity without being affected by the heavy metals. In other words, hyperaccumulators are plants that have a shoot-to-root metal concentration ratio greater than one. Phytoremediation can be used to clean up both organic and inorganic contaminants. The categories of phytoremediation to remediate organic contaminants are rhizofiltration, phytostabilization, phytovolatilization, rhizodegradation and phytodegradation whereas those for inorganic contaminants are phytoaccumulation, phytostabilization, phytovolatilization and rhizofiltration (Tangahu et al., 2011).



a **b**

Figure 3.3: (a) The mechanism of heavy metal uptake by phytoremediation plants (b) Factors affecting the uptake mechanisms of heavy metal (Tangahu et al., 2011)

3.3.2.1 Phytoextraction

Phytoextraction is the process whereby hyperaccumulators are used to absorb metals from soil via the roots to the shoots without the plant itself being affected by the high metal concentration. Evapotranspiration helps the translocation of metals from roots to shoots during the phytoextraction process (Tangahu et al., 2011). In this technique, the hyperaccumulators are planted in the metal contaminated site; the shoots are harvested after a period of time when the plants must have taken up high concentration of metals into the shoots and are either disposed as hazardous waste or undergo recovery treatment (Evanko et al., 1997). Phytoextraction is a solar driven technique. This technique explores the ability of plants to take up, translocate and compress heavy metals from soil through their roots to shoots (Khalid et al., 2017). Hyperaccumulators degrade heavy metals by intracellular (storage of metals in their vacuoles) accumulation or through transformation by some enzymes. They concentrate heavy metals 100-1000 times more than nonaccumlators (Tangahu et al., 2011). *Sedum plumbizincicola* is a cadmium/zinc hyperaccumulator. The combination of biochar and *S. plumbizincicola* in a pot experiment by Li et al. (2018) has been shown to effectively increase the removal of Cd/Zn efficiently from contaminated soil.

Plants used for phytoextraction have the following attributes; (a) profuse root system, (b) tolerance to elevated concentration of heavy metals, (c) the shoots must have high accumulation capacity of heavy metals, and (d) fast growth with large biomass (Khalid et al., 2017).

3.3.2.2 Phytostabilization

Phytostabilization is the process of demobilizing, stabilizing and binding of metal contaminants in soil by root exudates in order to reduce the bioavailability of the metal contaminants. During this process, metal contaminants are immobilized via roots adsorption, roots absorption and accumulation, or precipitation within the root zone by root exudates (Tangahu et al., 2011). Phytostabilization prevents and limit the movement of heavy metals to uncontaminated sites but cannot reduce the concentration or remediate the contaminated soil (Khalid et al., 2017). Phytostabilization employs plants that restrict the bioavailability and mobility of metals in the contaminated soil. The phytostabilizers have special characteristics that make them suitable to survive in the contaminated site; they have high tolerance of metals and accumulate low amounts of metals in their tissues. Phytostabilization has been used as a temporal strategy for containment while waiting for a better remediation technique.

Table 3.1: Plants that perform phytoextraction of heavy metals and metal contents in leaves (Vasilev et al., 2003, Marques et al., 2009)

Hyperaccumulator	Heavy metals	Metal content in leaves (mg/kg D.W)
Berkheya coddi	Ni	11,600
Sebertia acuminata		26,00
Minuartia vernia	Zn	11,400
Thlaspi caerulescens		39,600
Ipomea alpine	Cu	12,300
Pandiaka metallorum		6270
Thlaspi caerulescens	Cd	1800
Pteris vittata	As	7000
	Cr	20,675
Haumaniastrum rubertii	Co	10232
Maytenus bureavania	Mn	33750
Alyxia rubricalis		14000
Agrostis tenuis	Pb	13490
America maritime		1600
Astragalus racemosus	Se	14920
Psycotria vanbermanni	Ni	35720
Garcinia bakeriana		7440

3.3.2.3 Phytovolatilization

Phytovolatilization uses plants to take up heavy metals from polluted soil and transform them into gases which are then release into the atmosphere as biomolecules through transpiration. The concentration of the volatilized form of the contaminants is low and relatively non-toxic. Examples of plants that perform phytovolatilization are *Arabidopsis halleri, Brassica juncea, and Chara canescens* (Khalid et al., 2017, Tangahu et al., 2011).

3.3.2.4 Chelate assisted phytoremediation

Due to the limitation of phytoextraction by the low metal availability, uptake and translocation, and biomass, phytoextraction cannot be fully effective. Chelate assisted phytoremediation used both plants and chelating agents to remediate heavy metal contaminated soil effectively. This technique has received more attention and practice over the past 10 years and it is an economical alternative to conventional remediation techniques. Chelating agents has enhanced the phytoextraction of many heavy metals such as Cd, Zn, Pb, Cu and Ni. Example of chelating agents used in chelate assisted phytoremediation include humic substances, nitrate triacetic acid,

EDTA, hydroxyethylene diamine triacetic acid (HEDTA), EDDS, sulfur, and ammonium fertilizers (Khalid et al., 2017, Khanet al., 2000). The phytoextraction of Cd was enhanced by EDTA using *Lonicera japonica and Althaea rosea* plants in the black see region of turkey (Cay et al. 2016).

Table 3.2: Hyperaccumulators used in Phytoremediation (Omena et al., 2017)

Hyperaccumulator	**Heavy metals**
Berkheya coddi, Alyssum morale	Ni
Helianthus annuus, Minuartia vernia	Pb, Cd and Zn
Arabidopsis halleri	Cd and Zn
Astragalus racemosus	Se
Euphorbia cheiradenia	Cu, Fe, Pb and Zn
Pteris vittata, Eichhornia crassipes	As and Hg

3.3.2.5 Microbial assisted phytoremediation

Like chelate-assisted phytoextraction, microbial-assisted phytoextraction utilizes microorganisms to induce the reduction, absorption, oxidation and precipitation of heavy metal in the soil. Microbes have been known to increase the uptake of metals by hyperaccumulators. This technique uses various mechanisms such as redox reactions, biosorption, enzyme-catalyzed transformation, intracellular accumulation, bioleaching and biomineralization for the remediation of contaminated soil. Microbes lower soil pH, alters the redox condition of soil, produce plant growth promoting chemicals and metal chelating agents (examples are organic acids, siderophores and biosurfactants) in order to enhance the bioavailability and mobility of heavy metals in soil. *Bacillus mucilaginosus, Azotobacter chroococcum and Bacillus megaterium* secretes low molecular weight organic acids (LMWOAs) that lower the soil pH which facilitate the uptake of Zn, Pb and Cd by increasing their bioavailability (Khalid et al., 2017).

3.3.2.6 Advantages and limitations of phytoremediation

There are advantages of phytoremediation as well as disadvantages. Figure 3.4 shows the various advantages and disadvantages of using this technique to clean up heavy metal contaminated soils.

3.3.3 Biochemical Processes

Metals can be remediated from contamination sites by microbial induced oxidation and reduction reactions. In this technique, some microbes have direct influence to oxidise or reduce metals in contamination soil while others indirectly oxidise or reduce metal contaminants by producing chemical agents that initiate the redox process. Microbial mediated oxidation processes has been used to oxidize mercury and cadmium in contaminated soil and through microbial mediated reduction arsenic and iron have been reduced. This microbial assisted redox processes are being used to decrease or increase the mobility of metals by influencing the oxidation state of the metals (Evanko et al., 1997).

There are many important criteria involve in the process of selecting techniques for soil remediation. They include: (i) cost involved, (ii) time required, (iii) effectiveness under high metal(loid)s contamination, (iv) general acceptance and commercial availability, (v) long-term effectiveness, and (vi) applicability to multi-metal contaminated sites (Khalid et al., 2017).

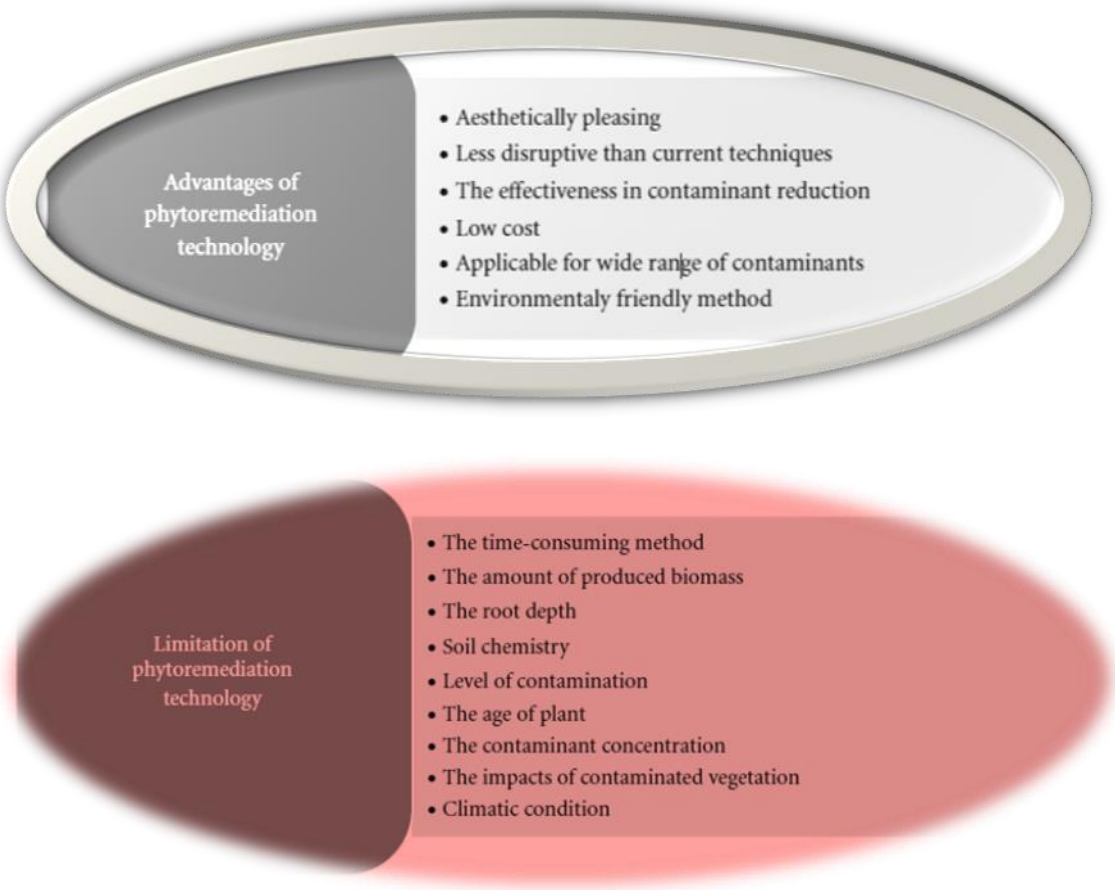


Figure 3.4: Advantages of phytoremediation and limitation of phytoremediation (Tangahu et al., 2011)

4. ANALYTICAL TECHNIQUES FOR HEAVY METALS DETERMINATION

The analytical techniques used for heavy metal determination include; atomic absorption spectroscopy (AAS), X-ray fluorescence spectrometry (XRF), inductively coupled plasma/mass spectrometry (ICP-MS), atomic fluorescence spectrometry (AFS), neutron activation analysis (NAA), inductively coupled plasma optical emission spectroscopy (ICP-OES), d.c argon plasma multielement atomic emission spectrometry (DCP-MAES), inductively coupled plasma atomic emission spectroscopy (ICP-AES). AAS is the most widely used analytical technique to determine the concentration of heavy metal in soil (Soodan et al., 2014).

Inductively coupled plasma optical emission spectroscopy (ICP- OES): ICP-OES has the ability for the analysis of trace amount of metals and is widely used. The advantages of ICP-OES include; limited volume of samples, multielement analysis, measures at nanogram level. Greenfield and his associates were the first to use ICP as an excitation source for the determination of trace metals in 1965 (Soodan et al., 2014).

Inductively coupled plasma atomic emission spectroscopy (ICP-AES): ICP-AES was first use by Govindaraju and Mevelle for the analysis of rock samples. It is a multielement analytic technique. It has gain wide used for heavy metal determination in soil from different geographical areas (Soodan et al., 2014).

X-Ray fluorescence spectroscopy (XRF): XRF is based on atom-radiation interaction and is highly sensitive. In this method, a primary x-ray excitation source from either a radioactive source or X-ray tube strikes the sample, the atoms of the sample absorbed the x-ray as the x-ray transfers its energy to an innermost electron (the photoelectric effect) or the x-ray is scattered through the sample. The atoms are unstable and needs to return to its normal (stable) state. In the process of the atoms returning to its normal state, outer shell electrons are transferred to inner shells and this produces a characteristic x-ray due to energy lost. The innermost K and L shells are involved in XRF most of the time. Each metal give off x-rays with a specific characteristics (Jignesh et al., 2012).This process of emitting characteristic x-rays is known as X-ray Fluorescence. XRF is the technique of using x-ray fluorescence for detection. It was used by Bhuyian et al to analyse heavy metals in agricultural fields (Soodan et al., 2014).

There are three types of X-ray spectrophotometers; the wavelength dispersive X-ray fluorescence (WDXRF), Total Reflection X-Ray Fluorescence Spectroscopy (TXRF) and energy-dispersive X-ray fluorescence (EDXRF). XRF methods are used for non-destructive analysis of samples. They are convenient and fast techniques.

Metals in concentration of ppm can be detected by WDXRF and EDXRF methods. TXRF can measure metals in concentration of ppb because of its higher sensitivity (Jignesh et al., 2012).

4.1 Atomic absorption spectroscopy (AAS)

AAS is the most commonly used technique for the determination of heavy metals in the environment. AAS was developed by Alan Walsh and his team in 1954 for metal content analysis. The principle of AAS is that free atoms that are generated in the atomizer absorb radiation at a specific frequency. This radiation is absorbed by passing UV or Vis through a monoatomic particle medium such as gaseous Hg (Jignesh et al., 2012). The absorption of this radiation leads to electrons moving from lower energy state to higher energy levels. AAS is able to determine more than 50 metals in solution, less time consuming, convenient and accurate as compare to other spectroscopic methods. Standards of known concentrations are used to determine the concentration of metals in sample through calibration curves. AAS uses furnace and flame. Though many advanced techniques have come in place, AAS still has a wide use due to the ability to analyse any type of matrices (Soodan et al., 2014). The atoms of the sample absorbed ultraviolet or visible light and move to a higher electronic energy level. The absorption is directly proportional to the metal concentration and the concentration is determined by standard graph curves (Soodan et al., 2014).

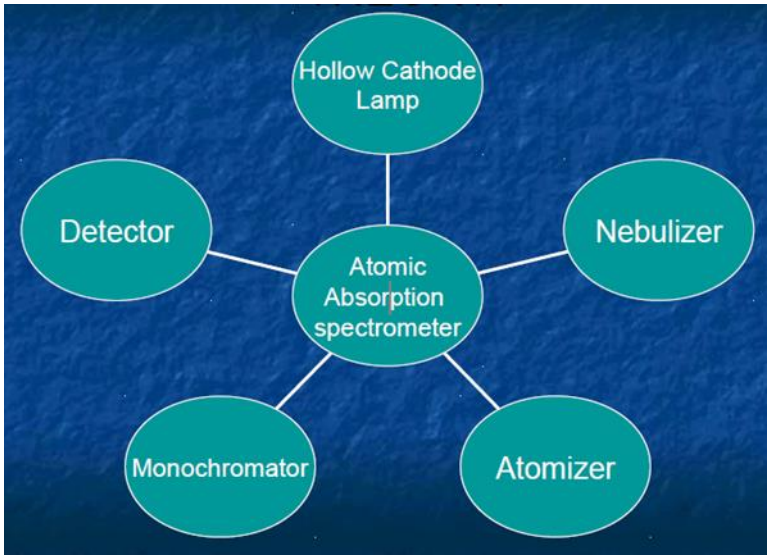


Figure 4.1: Theory of Atomic Absorption Spectroscopy

Radiation source (Hollow-Cathode Lamps, HCL)**:** HCL is the most common and is composed of a tungsten anode and a hollow cylindrical cathode. They are sealed in a glass tube containing an inert gas such as neon or argon at 1-5 torr pressure. Each metal has a unique lamp that is used during its analysis. Multielement cathode lamps are being used to determine more than one element (Jignesh et al., 2012).

Atomisation cell: The sample introduction is done at the atomisation cell where it is being dissociated for metal atoms to be released (Jignesh et al., 2012). Atomizer exposes the analyte to high heat in the flame or graphite furnace in order to separate the particles into atoms. The frequently used atomisation cell is a flame cell but graphite furnace is used for a higher sensitivity (Jignesh et al., 2012).

Flame Atomization Cell: In FAAS, a nebulizer is used to aspirate the liquid sample into a flame. The sample changes to a mist when in the nebulizer and they burn easily in the flame (Jignesh et al., 2012). Flame is created by mixing oxidant gas such as nitrous oxide acetylene and a fuel gas such as air-acetylene flame. The sample for flame atomizer is liquid or dissolved sample. FAAS is relatively inexpensive and simple to operate (Jignesh et al., 2012).

Limitations of flame atomic absorption spectroscopy:

i) The sample introduction system needs immense volumes of aqueous samples and it is also inefficient (Jignesh et al., 2012).

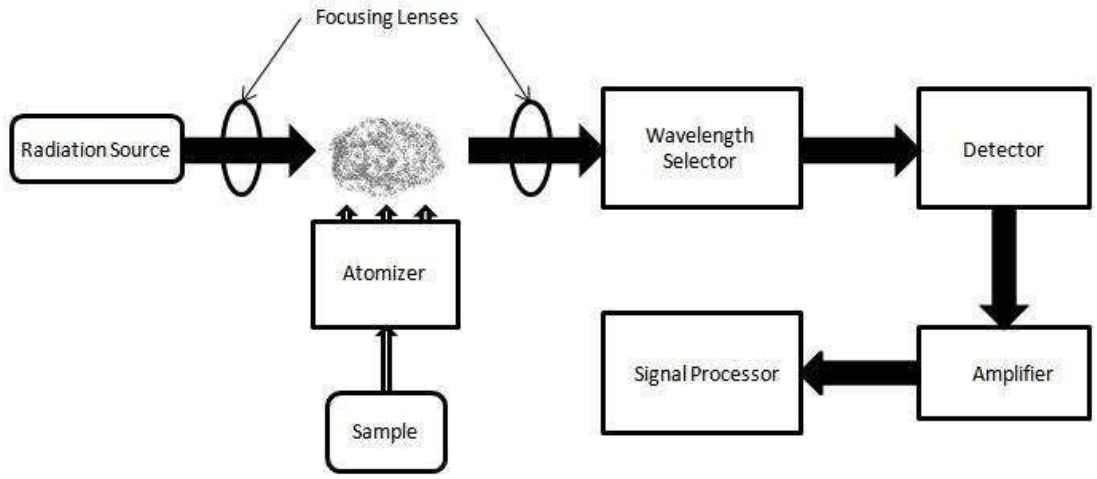
ii) The duration of the atom in the flame is limited due to the high burning velocity of the gases and this leads to a high limit of detection (Jignesh et al., 2012).

iii) Solid sample must go through dissolution process prior to analysis, thus it is unable to directly analyse solid samples (Jignesh et al., 2012).

Graphite Furnace (Electrothermal atomizer): The electrothermal atomizer has a cylindrical graphite tube which opens at both ends. The tube has a diameter of 3-8 mm and length of 5 cm. The sample is introduced in a central hole in the graphite coated tube by a micropipette and atomisation takes place in the graphite tube. The tube is heated by a high current power supply to be vaporised and atomised. GFAAS have good limit of detection for aqueous and solid samples than FAAS which makes it better than FAAS. GFAAS can also detect heavy metal levels of up to ppb and is very sensitive (Soodan et al., 2014).

Monochromator: Monochromator is very important for atomic absorption spectrometer and it is used as a selector. It selects a specific wavelength of light absorbed by the sample and excludes the others. This allows a particular metal to be determined amongst other elements.

Detector: A photomultiplier tube is the detector. It converts the light selected by the monochromator into an electrical signal proportional to the light intensity. The signal can be displayed for readout.



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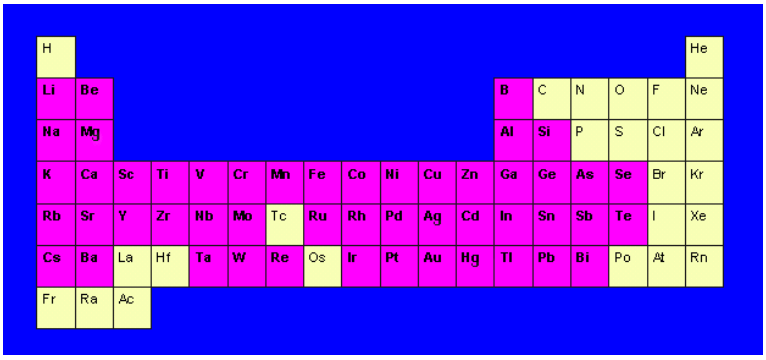

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Figure 4.2: (A) Block diagram of AAS (Jignesh et al., 2012); (B) Elements detectable by atomic absorption highlighted in pink

4.2 Inductively coupled plasma / mass spectrometry (ICP-MS)

ICP-MS is the best up-to-date technique for heavy metal determination with ultra-trace detection capability of multielements simultaneously (Soodan et al., 2014). This technique has a wide range of applications and it is more sensitive than the other techniques such as AAS, ICP-AES (Soodan et al., 2014).The sample is introduced into the nebulizer and the nebulizer converts the aqueous sample into an aerosol by the action of the carrier gas such as argon (Jignesh et al., 2012). The

generated aerosol is introduced into the spray chamber. The spray chamber further reduces the original aerosol particle size towards the ideal size by providing a surface for collisions and/or condensation. There are different types of nebulizer; Pneumatic concentric nebulizer, Cross flow nebulizer and Ultrasonic nebulizer (Jignesh et al., 2012). Argon is introduced to the ICP torch located in the centre of a radio frequency (RF) coil for energy supply. The RF field creates collision of Ar atoms which generate high energy plasma. The sample aerosol disintegrates in the plasma to form analyte atoms that are simultaneously ionized. The ions are transferred from the plasma into the mass spectrometer. ICP-MS was used by Alkas et al (2017) for the determination of heavy metals in biological samples and also by Soodan et al to monitor heavy metals in agricultural soil in Kocaeli city, Turkey (Soodan et al., 2014).

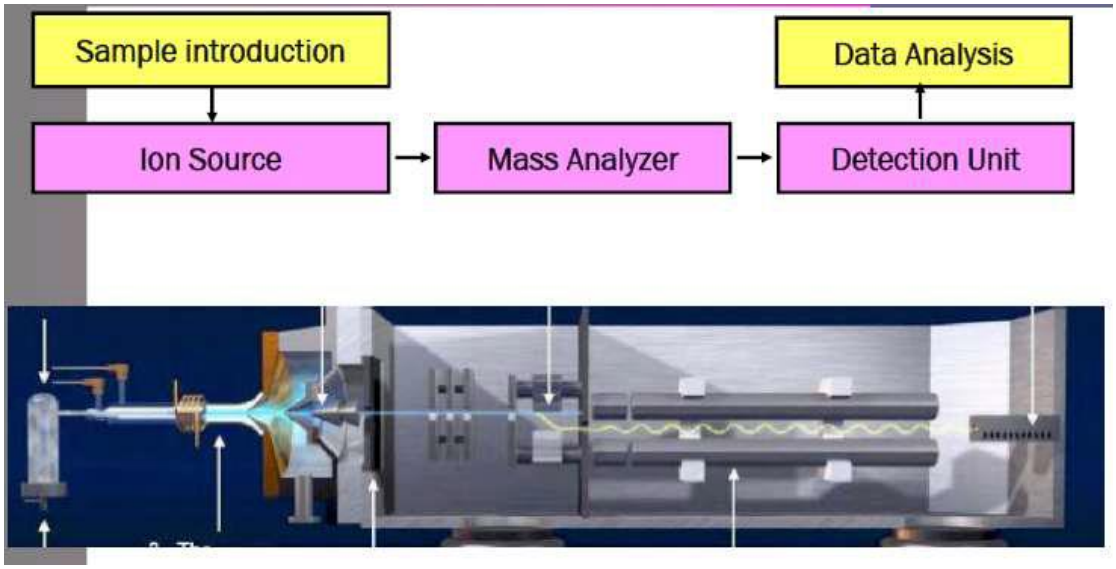

Figure 4.3: Illustration of ICP-MS components

ICP-MS is composed of nebulizer, inductively coupled plasma (a high temperature ionisation source, 8000 K), quadrupole mass spectrometer analyser, and detection unit. The coupling of the ICP torch that operates at atmospheric pressure with a mass spectrometer that operates under high vacuum is the major instrumental advancement that makes ICP-MS an efficient and major analytical technique. The quadrupole consists of 4 cylindrical rods of the same diameter (1 cm) and length (8-12 cm). When a direct current field is applied on a pair of rods and a RF field on the other opposite pair, the ions of selected mass pass through the rods to the detector whereas the ions are ejected from the quadrupole (Jignesh et al., 2012). In summary, ICP-MS operate on a mass-to-charge princiciple of separation.

There are many benefits of ICP-MS for the determination of metals as compare to other techniques; (i) it is very sensitive, (ii) both major and trace components can be estimated simultaneously, (iii) simple and complex sample matrices can be analysed, (iv) have extreme low limit of detection that range from ppb to ppt, and (v) measures individual isotopes (Jignesh et al., 2012).

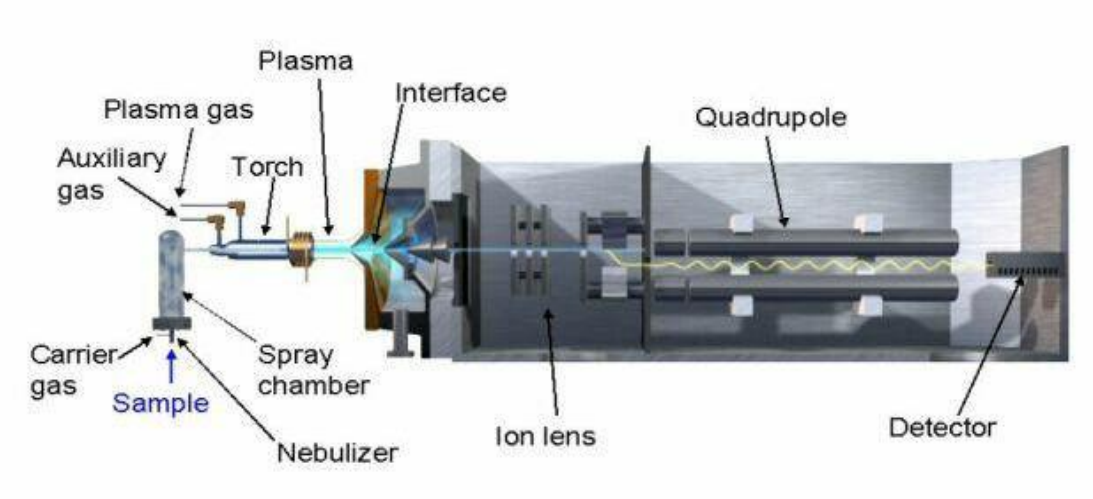

Figure 4.4: Schematic diagram of inductively coupled plasma-mass spectrometer

5. MATERIALS AND METHODS

5.1 Reagents, Chemicals and Apparatus

The following were used to carry out the analysis: nitric acid, purity 70%, and hydrochloric acid, purity 37% (Fluka, Madrid, Spain); hydrogen per-oxide solution for ultra-trace analysis, purity 35% (Sigma-Aldrich, Steinheim, Germany), microwave with model CEM Mars 5 (USA), Water was obtained from a Milli-Q^{TM} system (Millipore, Bedford, MA, USA), Whatman #42 filter paper (Merck, Darmstadt, Germany), Pyrex glass digestion tubes (Foss, MN, USA) and ICP-MS 7500ce (Tokyo, Japan)

5.2 Instrumentation

After the treatment of the soil and vegetable samples, they were analysed using Agilent 7500ce ICP-MS (Agilent Technologies, Japan). This 7500ce models has an ICP plasma-shielded torch, a concentric nebulizer, a quadrupole mass analyser and an octupole reaction system in a RF mode. The analysis was done in the following operating conditions; 99.99% spectral pure argon was used as nebulizer gas flow rate 0.9 L/min, as auxiliary gas flow rate 0.14 L/min, as plasma gas flow rate 15 L/min, helium as reaction gas flow rate 0.14 L/min, temperature of spray chamber 2 ^{o}C, and ICP RF power 1500 W. Table 5.1 below shows the validation parameters of the ICP-MS

Table 5.1: Validation parameters of the ICP-MS analysis

	Cr	**Mn**	**Ni**	**Cu**	**Cd**	**Pb**	**As**
Calibr. Range (ng/mL)	0-50	0-50	0-50	0-50	0-50	0-50	0-50
Determ. Coefficient (R2)	0.9998	0.9997	0.9999	1	1	0.9999	1
Rec.* (%)	91	99	89	95	110	93	94
RSD (%) (n=10)	6.9	7.8	10.2	9.9	10.1	11.9	12.4
LOD (µg/kg)	0.08	0.11	0.06	0.03	0.15	0.12	0.06
LOQ (µg/kg)	0.005	0.005	0.007	0.001	0.008	0.006	0.004

Rec. Recovery, RSD Relative standard deviation, LOD Limit of dedection, LOQ Limit of quantitation

5.3 Study Area

This study was carried out in Near East University, North Cyprus and Advanced Technology Education, Research and Application Centre Laboratory (ATERACL), Mersin University, Turkey. The areas of sampling were Gemikonagi (35°8′13.48″N, 32°49′57.41″E); that has an abandoned mine and tailings, and Dipkarpaz (35.617682°N, 34.408731°E) as the control site without any mining activities. The distance between this two selected areas are approximately 170 km.

Figure5.1: Gemikonagi (Karavostasi) and Dipkarpaz Location, Cyprus Map

5.4 Sample Collection, Pre-Treatment and Analysis

5.4.1 Soil Samples

On the 22nd of April 2018, 9 top soil (0-10 cm) samples each weighing 200 g were collected in pre-sanitised plastic zipper bags using a pre-sanitised PTFE-coated Scoop in the region of Gemikonagi. The soil samples were collected simultaneously with each vegetable sample at the same location. The sampling coordinates are shown in Table 5.2. The samples were kept at 4^{o} C to minimise bacterial colonisation and loss of moisture and transferred to the Near East University Toxicology Laboratory within 3 hours and the storage temperature was maintained

at 4^{o} C. 7 top soil samples were collected in labelled pre-sanitised zipper bags with PTFE-coated Scoop in Dipkarpaz and each soil sample weighed approximately 200 g. Each sample was collected beneath and around the vegetables. The samples were collected on the 29^{th} of April 2018 and transported to Near East University Toxicology Laboratory within 4 hours at 4^{o} C. Upon arrival at the laboratory, they were stored in the refrigerator at 4^{o} C until analysis. The sampling coordinates are shown in table 5.2.

Figure 5.2: Soil samples from Gemikonagi and Dipkarpaz

Prior to analysis, soil samples were kept at room temperature in a controlled area to air dry. After 72 hours, they were ground and sieved through a 1.0 mm sieve. The sieved samples were transported to Mersin University in labelled and tightly sealed pre-sanitized plastic bags. Upon arrival at ATERACL, 5 ml nitric acid (65%) was added to each tube containing 0.25g of soil sample. The mixtures were heated up to 180 ^{o}C until the acid was almost completely evaporated and the process was repeated twice. After the final heating process, deionised water was added and the suspension was filtered with whatmann filter (0.45 µm). The filtrate was made up to 50 mL with deionised water. This final filtrate was analysed using ICP-MS 7500ce model.

5.4.2 Vegetable Samples

Vegetable samples collected in the region of Gemikonagi were *Malva vulgaris* (Malva), *Lactuca sativa* (Lettuce), *Allium fistulosum* (Spring Onion), *Apium graveolens* (Celery), *Brassica oleracea* var. *botrytis* (Cauliflower), *Brassica oleracea* var. *italic* (Broccoli), *Brassica oleracea* var. *capitata* f. rubra (Purple Cabbage), *Brassica oleracea* var. *capitate* (Cabbage), and *Cynara scolymus* (Artichoke). They were collected with a stainless steel knife and sealed hermetically in pre-sanitised zipper bags, each weighing approximately 100 g. The vegetable samples were collected at the same time and location as the soil sample and their coordinates are shown on table 5.2. The samples were kept at 4^{o} C and transferred to the Near East University Toxicology Laboratory within 3 hours. Upon arrival at the laboratory, the samples were thoroughly washed with deionised water and rinsed again with deionised water and kept on clean surfaces for 4 hours to air dry. Then

they were sealed in pre-sanitised airtight polyethylene storage bags and stored at -20^{o} C until wet ashing treatment.

Figure 5.3: Vegetables samples from Gemikonagi and Dipkarpaz

Vegetable samples from Dipkarpaz were collected on the 29th of April 2018. 7 vegetable samples (malva, lettuce, spring onion, celery, purple cabbage, cabbage and artichoke) were collected in pre-sanitised polyethylene zipper bags each weighing 100 g at the same location and time of soil sample collection and transferred to the Near East University Toxicology Laboratory at 4^{o} C within 4 hours. Upon arrival at the laboratory, the vegetable samples were washed with deionised water, dead tissues excised and rinsed with deionised water. The washed samples were allowed to air dry for 4 hours and after sealed in pre-sanitised airtight polyethylene bags and stored in the freezer at -20^{o} C until treatment.

The vegetable samples were carried to ATERACL for analysis. A dual stage drying method was used to dry the sample to constant weight. Chopped vegetable samples were each put in crucibles and placed in an incubator for 30 minutes at 105 ^{o}C. At the second stage, the temperature of the incubator was set at 70 ^{o}C and the samples were allowed to dry for 12 hours (Zhou et al., 2016, Chang et al., 2013). The dried samples were ground, pulverised in an agate mortar and filtered with an 80 mesh sieve. 0.5 g of each sample was weighed and 30 ml of acid mixture (HNO3:HCLO4:H2SO4, 1:1:1) was added to each sample in an Erlenmeyer flask. They were kept in a fume hood for 24 hours. The mixtures were then heated at 90 ^{o}C on a hot plate until the volume reduced to 10 ml. An additional 10 ml acid mixture was added to the flask and heated to a final 4 ml volume. The flask was capped, allowed to cool at room temperature for an hour and 50 ml of deionised water was added to the flask. The sample mixtures were then filtered through a Whatmann #42 filter paper using vacuum assisted Buchner apparatus. Deionised water was added to the filtrate to have a 100 ml filtrate. ICP-MS 7500ce model was used to analyse the filtrate (Maleki et al., 2014).

Table 5.2: Location of sampling sites determined by global positioning system

S/N	Sample	Coordinates in Gemikonagi	Coordinates in Dipkarpaz
1	Malva; Soil	35°15'63"N, 32°80'99"E	35°59'86.39"N, 34°39'26.67"E
2	Lettuce; Soil	35°14'55.03"N, 32°85'56.52"E	35°59'86.39"N, 34°39'26.67"E
3	Spring Onion; Soil	35°14'55.03"N, 32°85'56.82"E	35°59'86.39"N, 34°39'26.67"E
4	Celery; Soil	35°14'55.03"N, 32°85'56.82"E	35°59'86.39"N, 34°39'26.67"E
5	Cauliflower; Soil	35°14'55.73"N, 32°85'66.25"E	/
6	Broccoli; Soil	35°14'55.73"N, 32°85'66.25"E	/
7	Purple Cabbage; Soil	35°14'62.76"N, 32°85'68.29"E	35°59'86.39"N, 34°39'26.67"E
8	Cabbage; Soil	35°14'57.57"N, 32°85'63.54"E	35°59'86.39"N, 34°39'26.67"E
9	Artichoke; Soil	35°14'69.09"N, 32°85'48.75"E	35°59'86.39"N, 34 °39'26.67"E

5.5 Data Analysis

Statistical analyses of the results were performed using PASW Statistics 18 (SPSS Inc, Hong Kong). Statistical significance level was accepted at $p < 0.05$.

Bioconcentration Factor (BCF) calculation

BCF was calculated as follows:

$$\mathrm{BCF} = \frac{C_{\mathrm{vegetable}}}{C_{\mathrm{soil}}}$$

Where Cvegetable is the total concentration of a particular heavy metal in the vegetable (mg/kg d.w), and Csoil is the corresponding heavy metal concentration in the soil habitat of the vegetable (mg/kg).

6. RESULTS

A total of 16 soil samples and 16 vegetable samples were analysed using ICP-MS. Gemikonagi has 9 soil samples and 9 vegetable samples while Dipkarpaz has 7 soil samples and 7 vegetable samples.

6.1. Heavy Metal Concentration in Sediment Samples

The metals analysed in the soil samples were Cd, Hg, Pb, As, Ni, Cr, Al, Mg, Fe and Cu. Their concentrations can be seen in the table below where Table 6.1 is the concentration of heavy metals in soil samples obtained from Gemikonagi, table 6.2 is soil metal concentration in Dipkarpaz.

6.2. Heavy Metal Concentration in Vegetable Samples

The concentrations of heavy metals in vegetable samples were given in Table 6.3 and Table 6.4 for Gemikonagi and Dipkarpaz respectively with their mean concentrations.

6.3 Bioconcentration of heavy metals from soil to vegetables

The BCF values of the different heavy metals in vegetable samples were calculated and the data were given in Table 6.5 and Table 6.6.

Table 6.1: Concentrations (ppm dry weight) of heavy metals in soil from Gemikonagi

HM	Sediment samples (ppm)									Mean	Min	Max
	GS1	GS2	GS3	GS4	GS5	GS6	GS7	GS8	GS9			
Cd	ND	ND	ND	ND	ND	ND	ND	ND	52.77	5.86	0.00	52.77
Hg	ND	ND	ND	ND	ND	ND	ND	ND	27.19	3.02	0.00	27.19
Pb	ND	ND	ND	ND	486.03	486.03	ND	ND	ND	108.01	0.00	486.03
As	ND	859.82	859.82	859.82	ND	ND	231.95	294.88	288.64	377.21	0.00	859.82
Ni	6200.00	19736.54	19736.54	19736.54	32045.64	32045.64	25604.12	22062.17	6671.92	20426.57	6200.00	32045.64
Cr	5953.43	18134.15	18134.15	18134.15	22629.97	22629.97	26521.9	18269.78	4725.31	17236.98	4725.31	26521.90
Al	44432.85	53264.14	53264.14	53264.14	47582.69	47582.69	57584.29	48832.71	52012.47	50868.90	44432.85	57584.29
Mg	25145.69	40296.18	40296.18	40296.18	38714.02	38714.02	49001.9	43552.39	37441.07	39273.07	25145.69	49001.90
Fe	59812.85	54605.81	54605.81	54605.81	52611.91	52611.91	55541.14	51931.28	57605.2	54881.30	51931.28	59812.85
Cu	253.18	182.42	182.42	182.42	169.83	169.83	202.68	177.67	186.69	189.68	169.83	253.18

HM is Heavy metal, ND is Not detected and GS is Gemikonagi sediment

Table 6.2: Concentrations (ppm dry weight) of heavy metals in soil from Dipkarpaz

HM	Sediment samples (ppm)							Min	Max	Mean
	DS1	DS2	DS3	DS4	DS5	DS6	DS7			
Cd	ND	ND	ND	ND	ND	ND	ND	/	/	/
Hg	ND	ND	ND	ND	ND	ND	ND	/	/	/
Pb	ND	ND	ND	ND	ND	ND	ND	/	/	/
As	1399.16	1085.49	977.17	668.13	762.92	674.41	1214.58	668.13	1399.16	968.84
Ni	33793.29	29780.55	27294.15	24034.84	25390.13	24109.28	24441.87	24034.84	33793.29	26977.73
Cr	34445.25	33363.04	23981.95	22692.74	22806.01	18855.36	19697.76	18855.36	34445.25	25120.30
Al	39242.27	37362.19	34501.42	30396.74	37445.41	33322.79	31683.88	30396.74	39242.27	34850.67
Mg	15331.81	15185.51	12361.52	12881.83	13984.68	12724.21	13053.62	12361.52	15331.81	13646.17
Fe	36265.91	36994.95	32115.23	42802.61	31990.34	29812.45	31627.52	29812.45	42802.61	34515.57
Cu	56.13	47.44	38.79	43.36	37.92	37.69	42.93	37.69	56.13	43.47

DS is Dipkarpaz sediment

Table 6.3: Gemikonagi metal concentrations (ppm dry weight) in vegetable samples

Vegetable samples	Heavy Metals									
	Cd	Hg	Pb	As	Ni	Cr	Al	Mg	Fe	Cu
Malva	19.23	ND	ND	0.76	ND	11.60	128.49	4247.44	175.65	7.44
Lettuce	12.70	ND	ND	ND	ND	43.76	5.46	512.39	21.21	2.40
Spring Onion	0.38	ND	ND	ND	ND	0.83	ND	288.57	2.26	0.79
Celery	7.73	ND	ND	ND	ND	42.55	6.49	1678.71	23.14	2.28
Cauliflower	0.73	ND	ND	ND	ND	7.96	ND	331.49	7.36	0.56
Broccoli	1.48	ND	ND	ND	ND	0.62	5.83	605.57	16.69	0.88
Purple Cabbage	2.91	ND	ND	ND	ND	13.20	ND	850.44	7.12	0.77
Cabbage	3.38	ND	ND	ND	ND	25.63	4.50	875.71	713.48	3.42
Artichoke	11.41	ND	ND	ND	ND	ND	ND	613.25	5.68	0.49
Min	0.38	/	/	/	/	0.62	4.50	288.57	2.26	0.49
Max	19.23	/	/	0.76	/	43.76	128.49	4247.44	713.48	7.44
Mean	6.66	/	/	0.08	/	16.24	16.75	1111.51	108.07	2.11

Table 6.4: Dipkarpaz heavy metal concentrations (ppm dry weight) in vegetable samples

Samples	Heavy Metals (ppm)									
	Cd	Hg	Pb	As	Ni	Cr	Al	Mg	Fe	Cu
Lettuce	224.32	ND	ND	ND	ND	1025.93	2.17	414.4	15.79	2.19
Artichoke	7.26	ND	ND	ND	ND	82.34	ND	543.28	10.59	1.68
Celery	0.01	ND	ND	ND	ND	8.66	43.03	552.68	42.43	11.34
Purple Cabbage	ND	ND	ND	ND	ND	8.25	ND	219.41	4.12	0.32
Cabbage	ND	ND	ND	ND	ND	0.14	ND	227.98	42.48	0.45
Spring Onion	0.92	ND	ND	2.77	ND	36.97	ND	119.12	5.68	0.49
Malva	7.50	ND	ND	ND	ND	19.52	75.48	1349.29	82.41	2.88
Min	0.01	/	/	/	/	0.14	2.17	119.12	4.12	0.32
Max	224.32	/	/	2.77	/	1025.93	75.48	1349.29	82.41	11.34
X_m	34.29	/	/	0.40	/	168.83	17.24	489.45	29.07	2.76

Figure 6.1 showed the mean heavy metal concentrations in the vegetable samples obtained from both Gemikonagi and Dipkarpaz.

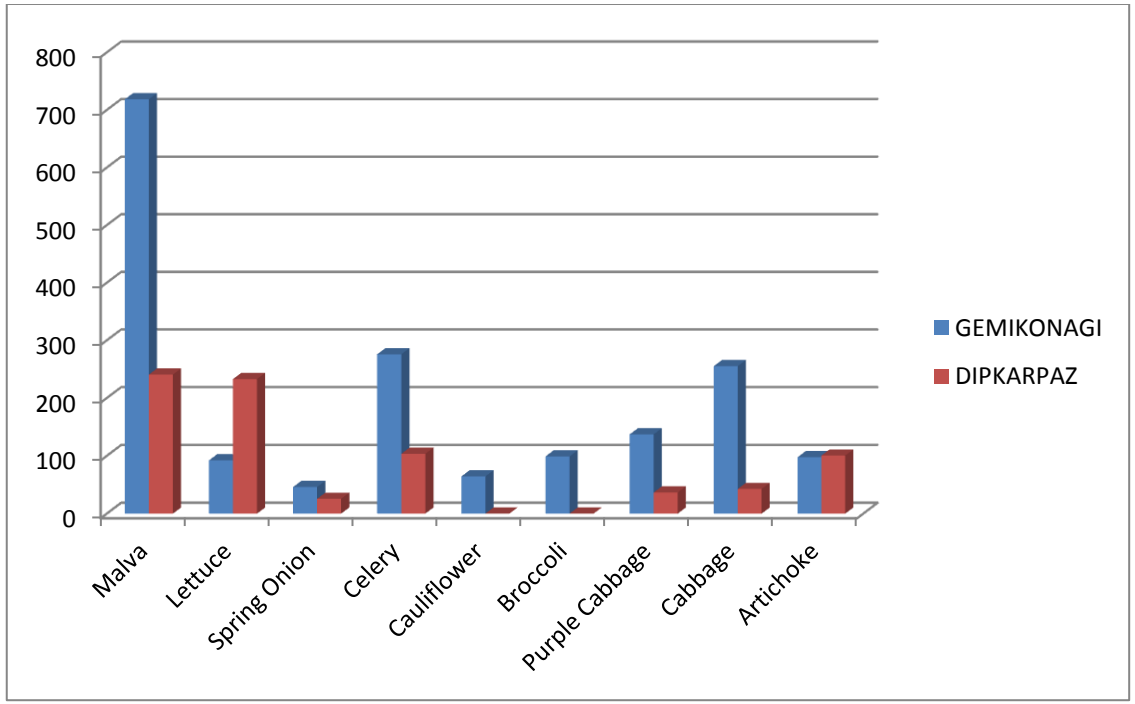

Figure 6.1: Comparison of vegetable sample mean concentrations of heavy metals

Table 6.5: The bioconcentration factor values of vegetables obtained from Gemikonagi

Heavy Metal	Bioconcentration factor (BCF)								
	Malva	**Lettuce**	**Spring Onion**	**Celery**	**Cauli-flower**	**Broccoli**	**Purple Cabbage**	**Cabbage**	**Artichoke**
Cd	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2162
Hg	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Pb	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
As	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Ni	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Cr	0.0019	0.0024	0.0000	0.0023	0.0004	0.0000	0.0005	0.0014	0.0000
Al	0.0029	0.0001	0.0000	0.0001	0.0000	0.0001	0.0000	0.0001	0.0000
Mg	0.1689	0.0127	0.0072	0.0417	0.0086	0.0156	0.0174	0.0201	0.0164
Fe	0.0029	0.0004	0.0000	0.0004	0.0001	0.0003	0.0001	0.0137	0.0001
Cu	0.0294	0.0132	0.0043	0.0125	0.0033	0.0052	0.0038	0.0192	0.0026

Table 6.6: The bioconcentration factor values of vegetables obtained from Dipkarpaz

Heavy Metal	Bioconcentration factor						
	Lettuce	Artichoke	Celery	Purple Cabbage	Cabbage	Spring Onion	Malva
Cd	/	/	/	/	/	/	/
Hg	/	/	/	/	/	/	/
Pb	/	/	/	/	/	/	/
As	0.0000	0.0000	0.0000	0.0000	0.0000	0.0041	0.0000
Ni	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Cr	0.0298	0.0025	0.0004	0.0004	0.0000	0.0020	0.0010
Al	0.0001	0.0000	0.0012	0.0000	0.0000	0.0000	0.0024
Mg	0.0270	0.0358	0.0447	0.0170	0.0163	0.0094	0.1034
Fe	0.0004	0.0003	0.0013	0.0001	0.0013	0.0002	0.0026
Cu	0.0390	0.0354	0.2923	0.0074	0.0119	0.0130	0.0671

6.4. Comparison of the Means and Standard Deviation (SD) of heavy metals with international and Turkish Maximum Permissible Limit (MPL)

The MPL set by World Health Organization (WHO) / Food and Agricultural Organization (FAO) and Turkish Soil Pollution Control Regulation (TSPCR) were compared to that obtained from this research as given in Table 6.7 and table 6.8 for vegetable samples and sediment samples respectively.

Table 6.7: Mean concentrations and SD of Metals in Vegetable Samples

Heavy Metal	Region	Mean ± SD	FAO/WHO MPL (ppm)
Cd	Gemikonagi	6.66 ± 6.56	0.10
	Dipkarpaz	34.29 ± 83.86	
Hg	Gemikonagi	0.00 ± 0.00	0.03
	Dipkarpaz	0.00 ± 0.00	
Pb	Gemikonagi	0.00 ± 0.00	0.30
	Dipkarpaz	0.00 ± 0.00	
As	Gemikonagi	0.08 ± 0.25	0.01
	Dipkarpaz	0.40 ± 1.05	
Ni	Gemikonagi	0.00 ± 0.00	67
	Dipkarpaz	0.00 ± 0.00	
Cr	Gemikonagi	16.24 ± 17.26	0.2
	Dipkarpaz	168.83 ± 378.96	
Al	Gemikonagi	16.75 ± 42.00	-
	Dipkarpaz	17.24 ± 30.20	
Mg	Gemikonagi	1111.51 ± 1246.41	-
	Dipkarpaz	489.45 ± 414.38	
Fe	Gemikonagi	108.07 ± 233.51	425
	Dipkarpaz	29.07 ± 28.55	
Cu	Gemikonagi	2.11 ± 2.24	73
	Dipkarpaz	2.76 ± 3.91	

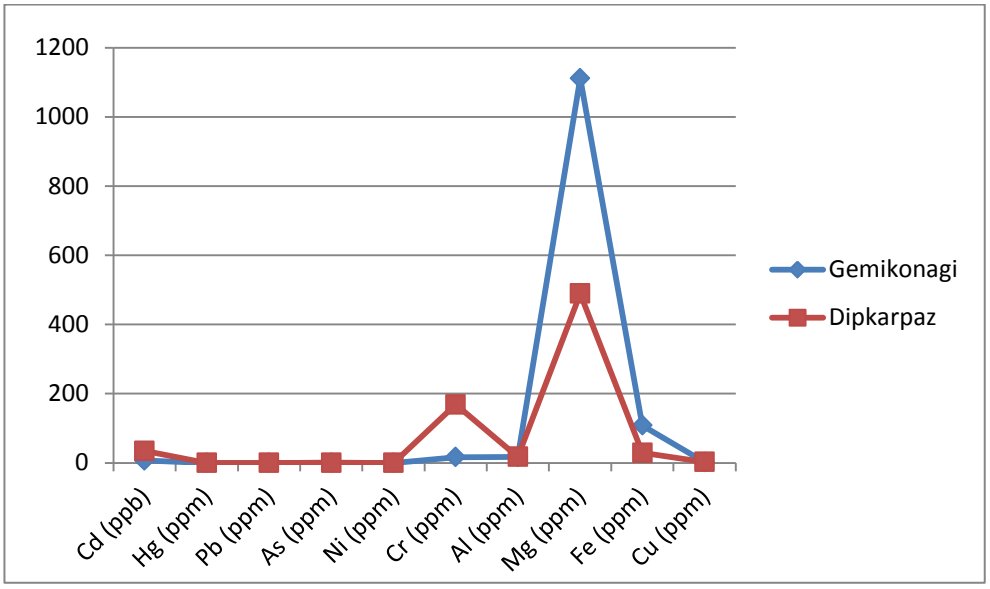

Figure 6.2: Mean Concentration of Heavy metals in Vegetable samples

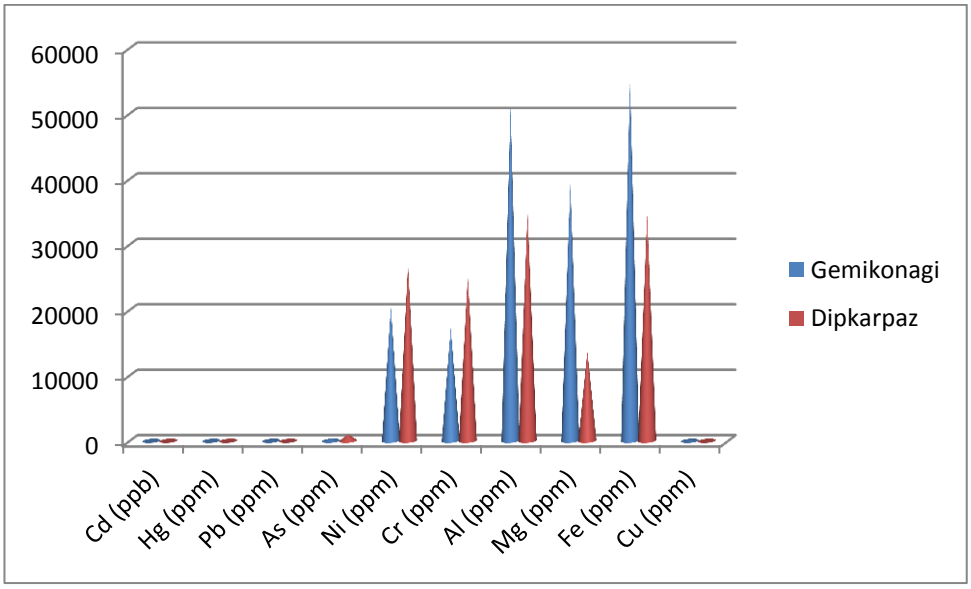

Figure 6.3: Mean Concentration of Heavy metals in Sediment samples

Table 6.8: Mean Concentration of Heavy metals in Sediment samples

Heavy Metal	Region	Mean ± SD	FAO/WHO MPL ppm	TSPCR 2001 (PH≥ 6) ppm
Cd	Gemikonagi	5.86 ± 17.59	3	3
	Dipkarpaz	00.00 ± 0.00		
Hg	Gemikonagi	3.02 ± 9.06	0.5	1.5
	Dipkarpaz	00.00 ± 0.00		
Pb	Gemikonagi	108.01 ± 214.32	100	300
	Dipkarpaz	00.00 ± 0.00		
As	Gemikonagi	377.21 ± 381.01	20	20
	Dipkarpaz	968.84 ± 282.48		
Ni	Gemikonagi	20426.57 ± 9303.91	50	75
	Dipkarpaz	26977.73 ± 3657.13		
Cr	Gemikonagi	17236.98 ± 7346.09	100	100
	Dipkarpaz	25120.30 ± 6272.90		
Al	Gemikonagi	50868.90 ± 4043.25	-	-
	Dipkarpaz	34850.67 ± 3282.50		
Mg	Gemikonagi	39273.07 ± 6321.27	-	-
	Dipkarpaz	13646.17 ± 1208.39		
Fe	Gemikonagi	54881.30 ± 2534.90	50000	-
	Dipkarpaz	34515.57 ± 4481.16		
Cu	Gemikonagi	189.68 ± 25.74	100	140
	Dipkarpaz	43.47 ± 6.61		

7. DISCUSSION

The high rate of industrialisation is a good move towards civilisation but it also has its consequences such as a source for heavy metal pollution in the environment which has a detrimental effects starting from the soil and air to plants and to humans. The natural occurrence of heavy metals and their natural concentration has little or no effect on the environment. Anthropogenic activities such as mining, energy industry (e.g.; biodiesel, petroleum, nuclear power and oil shale industry), agriculture (e.g.; sewage sludge irrigation, fertilizers, and pesticides), manufactured products (e.g paints, detergents, leathers and inks) has contributed so much to the mobilisation of heavy metals in the environment. The presence of heavy metals in the environment above maximum allowable limit can cause soil barrenness, many toxic effects in plant such as plant growth inhibition, production of ROS in their tissues, inhibition of photosynthesis and chlorosis, and also toxic effects in humans after consumption of plants (itai itai Japan 1912), fish (minamata disease in Japan 1956), or oil (toxic oil syndrome in Spain 1981).

In this study carried out in two regions of Cyprus, the region of Gemikonagi which has a history of mining and also subsistence farming was compared to another region, Dipkarpaz with no mining or pollution history but has subsistence farming activities. Due to the lack of national standards for heavy metal concentration in soil and vegetable in north Cyprus, the values were compared to that of Turkey, and WHO/FAO.

7.1. Heavy Metals in Vegetable

The various metals analysed were constant in all the samples in both Gemikonagi and Dipkarpaz. Malva was found to have the highest levels of heavy metals in both regions. The order of heavy metal accumulation by the vegetables in Gemikonagi were malva > celery > cabbage > purple cabbage > broccoli > artichoke > lettuce > cauliflower > spring onion whereas in Dipkarpaz were malva > lettuce > celery > artichoke > cabbage > purple cabbage > spring onion. From this irregular pattern of heavy metal accumulation by the vegetables in the two regions, the chemical and physical characteristics of the soil were different in the regions hence different accumulation pattern because the cation exchange capacity, soil organic matter, pH, clay content and water content affects the uptake of heavy metals by plants.

The vegetable samples from Gemikonagi had the highest mean concentration of heavy metals as compare to Dipkarpaz and the level in Gemikonagi (Malva 718.53 ppm) almost triple that in Dipkarpaz (Malva 240.47 ppm). From this result, the hypothesis that Gemikonagi will have higher level of heavy metals due to the tailing and abandon mining facility than Dipkarpaz is true. Amongst the vegetable samples from Gemikonagi, the vegetable with least mean heavy metal concentration was spring onion (45.96 ppm) while malva (718.53 ppm) was the highest and from

Dipkarpaz spring onion (25.55 ppm) was the least as well as malva (240.47 ppm) with the highest mean heavy metal concentration as shown in Figure 7.1.

The concentration of heavy metals in the vegetable sample showed a wide variation. The metals analysed in vegetable samples obtained from Gemikonagi were Cd (min:0.38 – max:19.23 ppm), Hg (not detected), Pb (not detected), As (not detected – 0.76 ppm), Ni (not detected), Cr (not detected – 43.76 ppm), Al (4.50 – 128.49 ppm), Mg (288.57 – 4247.44 ppm), Fe (2.26 – 713.48 ppm) and Cu (0.49 – 7.44 ppm). The analysed metal from Dipkarpaz included Cd (0.01 – 224.32 ppm), Hg (not detected), Pb (not detected), As (not detected – 2.77 ppm), Ni (not detected), Cr (0.14 – 1025.93 ppm), Al (2.17 – 75.48 ppm), Mg (119.12 – 1349.29 ppm), Fe (4.12 – 82.41 ppm) and Cu (0.32 – 11.34 ppm).

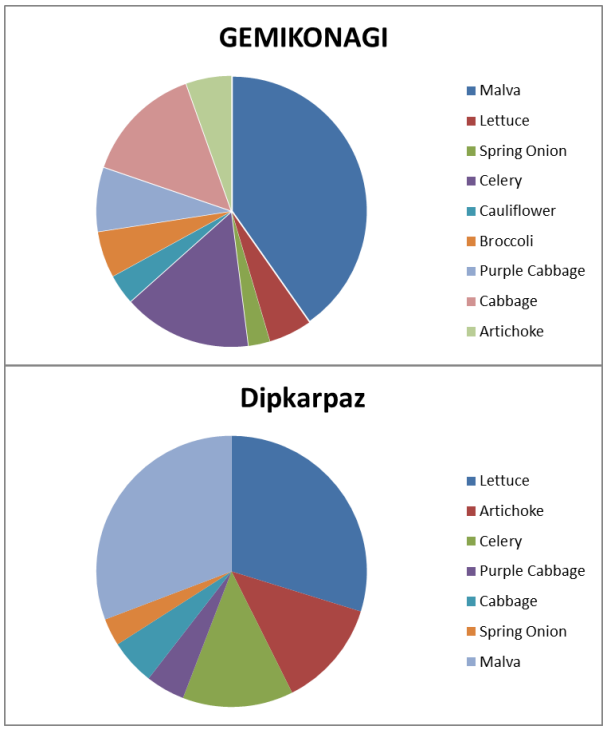

Figure 7.1: A pie chart of heavy metal concentration in vegetables

In this present study, Mg was found with the highest concentration in both regions while Cd had the least concentration. The mean concentration of Cu and Fe of vegetable sample in both regions were below the WHO/FAO standards. The mean concentration of Cd and Cr were far above the maximum permissible level set by WHO/FAO and this might be primarily due to the abandon mine site in Gemikonagi and human activities such as fertilizers application or use of sewage water in

agricultural soils which increase the heavy metal levels in Dipkarpaz. The level of As was slightly above the maximum permissible level in both regions.

7.2 Heavy Metal Concentration in Soil

Generally, soil organic matter, clay content, cation exchange capacity, electrochemical conductivity and soil pH greatly influence the concentration of heavy metals in soil and the bioavailability and bioaccumulation in plants. There were 10 heavy metals which were analysed in the soil samples and these are the metals in increasing order of mean concentration in Gemikonagi Hg < Cd < Pb < Cu < As < Cr < Ni < Mg < Al < Fe and in Dipkarpaz Cu < As < Mg < Cr < Ni < Fe < Al. Three heavy metals (Hg, Cd and Pb) were not detected in the soil samples from Dipkarpaz. Among the detected metals in the soil samples, the concentration of Fe was the highest and the least concentration was Hg in the soil samples from Gemikonagi whereas in Dipkarpaz the highest was Al and the lowest was Cu. This irregularity in the trend of metal concentration in the two regions was as a result of the difference in the chemical and physical property of the soil and the area of sample collection in Gemikonagi was closed to the sea while that of Dipkarpaz was further away. The concentration of heavy metals in soil samples were compared to the maximum permissible level of World Health Organization and Turkish Soil Pollution Control Regulation (TSPCR). In Dipkarpaz, the concentration of Fe and Cu were below the MPL of WHO AND TSPCR whereas the concentration of As, Ni, and Cr were above the MPL of both. In Gemikonagi, none of the heavy metals were below the MPL of WHO, however Pb was found to be below the MPL of TSPCR and slightly above the MPL of WHO. The mean concentrations of Cd, Hg, and Fe in soil from Gemikonagi were slightly above the MPL of both WHO and TSPCR.

7.3 Bioconcentration

The physio-chemical properties of soil such as texture, moisture, organic matter, pH and the cation exchange capacity (CEC) of soil greatly influence the form of the metals and their uptake into plants. Plants with a bio-concentration factor more than 1 are termed hyper-accumulator and those with a factor below 1 are non-accumulators. By the above classification, none of the vegetables were bioaccumulator as the highest BCF values were 0.2923 of Cu in Celery from Dipkarpaz and 0.2162 of Cd in artichoke from Gemikonagi. The lowest bio-concentration factor was 0.0001 in both regions of Fe in artichoke, purple cabbage, and cauliflower and for Al in lettuce, celery, broccoli, and cabbage. Notably, the BCF was 0.0001 of Fe in purple cabbage for both Gemikonagi and Dipkarpaz. The

above value showed that Celery takes up Cu more than other heavy metals whereas the uptake of Cd was more in artichoke. The very low bio-concentration values shown in table 6.5 and 6.6 in both regions indicated that the vegetables capacity to take up heavy metals from the soil is low and the physicochemical characteristics of the soil do not favour the uptake of heavy metals. The high transfer potential of Cd and Cu from soil to plants is attributed to the uptake mechanism which is similar to some other +2 essential elements such as Ca and Mg which are taken up by passive transport.

8. CONCLUSION

North Cyprus practice subsistence agriculture in its entire region and mostly in villages producing vegetables and fruits in large quantity to meet up with the everyday demand of the inhabitants. Increase cancer cases implies the presence of carcinogenic substances in the environment and a study by Akun et al (2011) showed high concentration of heavy metals such as Arsenic, Lead and Cadmium in soil which are all carcinogens. Majority of the heavy metals analysed were above the acceptable limit set by World Health Organization which indicated that large amount of heavy metals is ingested through food. The bioconcentration factor value indicated that Cd and Cu have a higher transfer potential from soil to vegetables. Spring onion had the lowest mean concentration of heavy metals while malva had the highest. To avoid the increase of metal contents in soil, sewage sludge and chemical fertilizer with heavy metal content should not be used for crop cultivation as the soil already contain unacceptable high level of heavy metals. A perfect suitable remediation technique should be done to clean up the heavy metal contaminated soils and the best, cheapest and eco-friendly technique should be phytoremediation which could be implemented by the government of North Cyprus or private organization.

Further research work will be carried out for the assessment of potential human health risk associated with food consumption using the Target Hazard Quotient (THQ).

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