ENVIRONMENTAL IMPACT ON ANTIOXIDANT ACTIVITY IN VEGETABLES

A DISSERTATION

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By

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CANDIDATE DECLARATION

I Astha Saxena hereby declare that the work being presented in the dissertation report entitled "**Environmental Impact and antioxidant activity in vegetables Plants"** is an authentic record of work carried out by me during the period July 26th 2011 to April 24th, 2012 at "Indian Institute of Technology, Roorkee (IIT, Roorkee)" under the guidance of **Dr. R.K DUTTA**, Astt. Professor. This is being submitted for the partial fulfillment for the (dissertation) award of Master's Degree (M.Tech) in Department of Chemistry (**Advanced Chemical Analysis**) of Indian Institute of Technology (IIT), Roorkee (U.K). I have not submitted the matter embodied in the dissertation report in any other institution for the award of any other degree.

DATE:

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CERTIFICATE

This is to certify that the above statement made by the candidate is correct to the best of my knowledge.

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Abstract

In this dissertation report our main objective is to study the Environmental Impact on Vegetable Plants mainly, comparison of metal amassment and Antioxidant activity of mining impact site sample and control site samples in vegetable plants. Metal detection was done by different techniques i.e. AAS, ICP-MS and Flame photometry and different Antioxidants assay was done for comparison.

CONTENTS

The basic need of human is food for which they relied on plants. Plants are grown in bio-friendly environment are in great demand in today's life because human body is more habituated to natural products. There are many constituents in plants that are compatible to human digestive system, which regulate its absorption and reduce the side effects [1, 2, 3].Vegetable plants are also very useful for strengthening immune system in humans and hence facilitate towards maintaining good health and longevity.

Nowadays Vegetable plants are used as home remedies, nutritional supplements to maintain good health and a disease free body. The chemical constituents of Vegetable plants consist of flavonols, alkaloids, proteins, amino acids, enzymes, aroma forming substances, vitamins and minerals [4, 5, 6, 7]. It has been also believed in ancient time that Vegetable plants also used for curative purposes and the efficiency of Vegetable plants for curative purpose primarily accounted in terms of its large number of bioactive compounds (organic constituents). One main reason for Vegetable plants are used for curative purpose is that they are economical and available in large amount for humans in every part of the world.

However, metals and metalloids present in Vegetable plants play important role in various physiological, biochemical and metabolic processes in humans [8, 9]. They referred to as essential elements. In addition, it is worth mentioning that certain elements which are not categorized as the essential elements, e.g.Ga [10], Pt [11], As [12], Hg [13], Pb [14] play important role in pharmaceutical drug formulations for therapeutic purpose in both contemporary as well as traditional medicine.

As stated above that human body requires number of essential inorganic elements or minerals in order to maintain good health, these are obtained mainly from food items. Fresh fruits and Vegetables are though high in vitamins and other important organic constituents related to health issues, they are often low in essential elements [15]. Edible plants, which accumulate minerals essential for their growth, can be considered to be natural source of mineral supplements to humans. Several studies have been carried out to estimate the concentration of essential and non-essential elements in large number of Vegetable plants [16, 17], in order to assess the parts of the plants that are suitable for elemental supplementation [18]. It has been argued that the trace element may need to be combined or chelated with some ligand (e.g. porphyrin), in order to be physiologically absorbed and thus be able to prevent or cure any impairment caused by the deficiency of an element. However a direct correlation between trace elements and therapeutic action in humans is yet to be established [19, 20].

1.2 Common Vegetable Plants and their function

1.2.1 Spinacia oleracea (Amaranthaceae)

Spinach is an annual plant, which grows to a height up to 30 cm. The leaves are alternate, simple, ovate to triangular-based, very variable in size from about 2–30 cm long and 1–15 cm broad, with larger leaves at the base of the plant and small leaves higher on the flowering stem. The flowers are inconspicuous, yellow-green, 3–4 mm diameter, maturing into a small, hard, dry, lumpy fruit cluster 5–10 mm across containing several seed.

Spinach (Spinacia oleracea) is an important dietary vegetable rich in antioxidants that is commonly consumed fresh in salads or after boiling fresh, frozen or canned leaves. Spinach contains several active antioxidant components, including flavonoids, p-coumaric acid derivatives, and uridine, which are reported to act synergistically [21, 22]. Spinach leaves contain about 1000–1200 mg kg of total flavonoids[23,24], and flavonoid levels have been shown to be affected by genetics [23,25], maturation [26,27], growing season [23], fresh-cut processing and domestic cooking [24], and frozen storage. At least 15 flavonoids consisting mainly of patuletin and spinacetin derivatives have been identified in spinach. These include the glucuronides and acylated di- and triglycosides of methylated and methylene dioxide derivatives of 6- oxygenated flavonols, Fig.1.1 (Annexure-I) [23-25, 29-32].

Flavonoids are known to display a wide array of pharmacological and biochemical actions[33]. Flavonoids and other phenolic compounds act as antioxidants by the free radical scavenging properties of their hydroxyl groups, and are also effective metal chelators. The extensive conjugation across the flavonoid molecule and numerous hydroxyl groups enhances their antioxidant properties, allowing them to function as reducing agents, hydrogen or electron-donating agents, or free radical scavengers [34]. Flavonoids also possess anti-allergic, anti-inflammatory, anti-thrombotic, anticarcinogenic and antiviral actions, which in part may be related to their free radical scavenging properties [33]. Spinach ranks high among vegetables in oxygen radical absorbing capacity (ORAC), an in vitro assay that measures the peroxyl scavenging capacity of plant extracts [35]. In addition, spinach flavonoids and water-soluble spinach extracts have been shown to have antimutagenic [36, 37] antioxidative [32,38], antitumor[39], and anti-inflammatory properties [40] in biological systems, but have no potential adverse estrogenic activity [41] or toxic effects in animals[42]. These studies suggest that spinach extracts may exert beneficial effects such as chemo- and central nervous system protection, and anticancer and antiaging functions [42, 43].

1.2.2 Brassica oleracea (brassicaceae)

Cabbage is a leafy green vegetable. It is herbaceous, biennial, dicotyledonous flowering plants distinguish by a short stem upon which crowded a mass of leaves, usually green but in some varieties red and purplish, which while immature form a characteristic compact, globular cluster (cabbagehead). Leaves are close together, round, smooth and slightly notched at the margins. Regarding the organoleptic properties, internal and external leaves are considerably different: internal leaves are pale yellow and are tender and sweeter than the dark green external leaves, which may influence the consumer's choice. Due to these characteristics internal leaves are eaten raw in salads or, most usually, cooked. As far as we know, only the phenolic composition of the external leaves has been described, consisting of complex flavonol glycosides [44] and nothing has been reported about the antioxidant capacity of tronchuda cabbage.

The phenolic composition of tronchuda cabbage leaves has already been reported: the external leaves were characterized by the presence of complex flavonol glycosides [45], while the internal ones exhibited both flavonol glycosides and hydroxycinnamic acid derivatives. The organic acids profile and the antioxidant capacity of external and internal leaves were also previously described [44, 59]], with the external ones exhibiting higher antioxidant potential. However, nothing has been reported about tronchuda cabbage seeds. In fact, several studies with other Brassica species have reported the existence of phenolics in the seeds, namely phenolic acids and their derivatives [57, 58,], flavonoid glycosides [57] and tannins. These compounds have been considered as UV screens in young seedlings [60] and have been associated with seedling vigour, height and weight [61, 62]. Regarding B. oleracea seeds, previous studies with different varieties, other than costata, concerned its germination sensitivity to hypoxia , the effect of its film-coating to control insect pests, the determination of glucosinolates [63] and fatty acids [56] and sterols .

Brassicaceae vegetables are reported to reduce the risks of some cancers, especially due to their contents of glucosinolates and derived products [52, 54]. Flavonoids and other phenolics are also considered to contribute to this capacity [47, 48, 55]. The antioxidant activity of some Brassica oleracea varieties has already been investigated. Cauliflower has been assayed for the ability to scavenge DPPH and ABTS+, as well as for ferric reducing efficiency and ability to inhibit lipid peroxidation [50]. The oxygen radical absorbance capacity values have been measured in broccoli [49, 51]. To find the antiradical activity of white cabbage, hydroxyl radical was used [53].

1.2.3 Trigonella foenum-graecum (Fabaceae)

Fenugreek is a annual green leafy vegetable. Fenugreek has three culinary uses: as a herb (dried or fresh leaves), as a spice (seeds), and as a vegetable (fresh leaves, sprouts, and microgreens).The destinctive cuboid yellow to amber coloured fenugreek seeds are frequently encountered in the cuisine of the Indian subcontinent. The seeds are used in the preparation of pickles, vegetable dishes, daals, and spice mixes, such as panch phoron and sambar powder. Fenugreek seeds are used both whole and in powdered form and are are often roasted to reduce their bitterness and enhance their flavor.Dried fenugreek leaves, called kasuri methi (or kasoori methi or qasuri methi) in North India and Pakistan, after the region of Kasur (Qasur) in Punjab, Pakistan province, where fenugreek grows abundantly, are used as an herb in a wide variety of dishes and breads. The dried leaves have a somewhat bitter taste and a characteristically strong but pleasant fragrance. Fenugreek is also used as a vegetable. Fresh fenugreek leaves are a main ingredient in many Indian curies. The sprouted seeds and microgreens are used in salads

Fenugreek (Trigonella foenum-graecum) being rich in phytochemicals has traditionally been used as a food,forage and medicinal plant [65,66].Fenugreek seeds contain lysine and L-tryptophan rich proteins, mucilaginous fibre and other rare chemical constituents such as saponins, coumarin, fenugreekine, nicotinic acid, sapogenins, phytic acid, scopoletin and trigonelline, which are thought to account for many of its presumed therapeutic effects [67]. Various components of the seeds have varying activities. For example, the component called fenu-greekine, a steroidal sapogenin peptide ester has hypo- glycemic properties [68]. It is shown to delay gastric emptying, slow carbohydrate absorption, and inhibit glucose transport in humans [69]. It can increase the erythrocyte insulin receptors and peripheral glucose utilization, thus showing improved pancreatic function. Trigonelline, another component is suggested to exert hypoglycemic effects in healthy patients without diabetes [68]. Thus the bestdocumented medical use of fenugreek is to control blood sugar in both insulin-dependent (type 1) and noninsulin - dependent (type 2) diabetics [70]. Treatment with fenugreek seed powder normalized the enhanced lipid peroxidation and increased susceptibility to oxidative stress associated with depletion of antioxidants in diabetic rats. In normal rats supple-mentation resulted in increased antioxidant status with reduction in peroxidation [71].

The steroidal saponins (diosgenin, yamogenin, tigogenin and neotigogenin) are thought to inhibit cholesterol absorption and synthesis and hence its potential role in arteriosclerosis. Clinical studies demonstrated a statistically significant decline in human serum total cholesterol, triglycerides and LDL cholesterol by fenugreek consumption [72-73]. It is also used topically to treat inflammation, and to promote postpartum lactation in animals.The beneficial gastroprotective effect of fenugreek seeds has been researched in gastric ulcers of rats [74]. There is considerable commercial interest in growing fenugreek for its high sapogenin content. At present diosgenin, a steroid sapogenin used in the manufacture of birth control pills is isolated from Dioscorea species. This is the starting compound for over 60% of the total steroids,

hormones and cortisone production by the pharmaceutical industry. Fenugreek being an annual and easy to cultivate might one day replace the present commercial sources. Plant phenolics have potential health benefits mainly due to their antioxidant properties such as reactive oxygen species (ROS) scavenging and inhibition, electrophile scavenging and metal chelation.[74]Epidemiological studies support a relationship between the consumption of phenolic rich food products and a low incidence of coronary heart disease [76], atherosclerosis[77], certain forms of cancer [78], and stroke [79]. They have also been reported to exhibit pharmacological properties such as antitumor, antiviral, antimicrobial, anti-inflammatory, hypotensive and antioxidant activity [80-81]. They are plant secondary metabolites, primarily synthesized through the pentose phosphate pathway (PPP), shikimate and phenyl-propanoid pathways. The oxidative PPP provides precursor erythrose-4-phosphate for the shikimate pathway. The shikimate pathway converts these sugar phosphates to aromatic amino acids like phenylalanine, which becomes the precursor for the phenylpropanoid pathway.

1.2.4 Brassica juncea (Brassicaceae)

Mustard is a annual green leafy vegetable. The leaves, the seeds, and the stem of this mustard variety are edible. B. juncea can hyperaccumulate cadmium and many other soil trace elements. Especially cultured, it can be used as selenium, chromium, iron and zinc food supplement. This plant is used in phytoremediation to remove heavy metals, such as lead, from the soil in hazardous waste sites because it has a higher tolerance for these substances and stores the heavy metals in its cells.

The use of metal-accumulating plants to remove toxic metals, including Cd, Zn and Cu [87] from soil and aqueous streams has been proposed as a possible solution to this problem [85]. This process of using plants for environmental restoration is termed "phytoremediation". Phytoremediation takes advantage of the ability of plants to adsorb, uptake and concentrate elements and organic compounds contaminating the environment. Phytoremediation has been viewed as a promising technique to remediate metal-contaminated soils because it offers advantages of being in situ, cost effective, and nondestructive [84]. In order to find suitable plants for removal Cd from the contaminated environment, we need a wide range of knowledge concerning the physiological and biochemical features of potentially useful species. Preliminary surveys for biomass, cadmium accumulation and membrane lipid composition of Brassica juncea, identified as a metal accumulator and a high biomass crop plant within the Brassicaceae family [83].

Cultivars of Brassica juncea (L.) Czern. (Indian mustard, a high biomass forages and oil crop) have also demonstrated the ability to accumulate as high as 1.5% Pb in shoot tissues when grown in nutrient solution with high concentrations of soluble Pb [86]. At lower Pb concentrations in solution, the shoot tissue accumulations were substantially less, although root

concentrations were very high. In spite of the significant capacity of B. juncea plants to concentrate Pb and translocate it to the shoots in solution culture, little uptake into the shoots was observed in B. juncea plants growing in soils where Pb bioavailability is limited [86]. In [82] paper, reported the enhanced uptake of Pb accumulation in soil-grown B. juncea plants with synthetic chelates.

1.3 Environmental Contamination and effect on vegetable plants

Environmental pollution is the contamination of environment which causes discomfort, instability, disorder and leaves harmful impact on physical system and on living organism. Pollution can take the form of chemical substances, or energy, such as noise, heat, or light energy. Pollutants, the elements of pollution, can be foreign substances or energies, or naturally occurring; when naturally occurring, they are considered contaminants when they exceed natural levels. With the increasing awareness day by day about the environmental degradation and pollution, the field of ecology has become an entirety in itself. Ecology is all about how environment is maintained, degrades, and destroyed by human beings and the various harmful effects that come associated with the ecological imbalance.

Most established vegetation within the vicinity or influence of industrial and urban areas is subjected to the regular or episodic impact of pollution .complete decimation of the vegetation as a consequence of this is a relatively infrequent occurrence, although mining and industrial wastes containing metals or other toxins may remain virtually devoid of vegetation [88]. The acute phytotoxic effects of many environmental contaminants are well known, but studying the possible chronic effects of lower concentration of atmospheric contaminants on growth and vigour of plants is probably of more relevance, particularly to long-lived plants. This has been a major focus of research in European and North American forests (Pitelka,1988;Schulze,1989) but much evidence is speculative due to the lack of long term data(Blank et al., 1989).

In many herbaceous plants the selection pressure of pollution has led to the natural evolutionary development of tolerant plant geneotypes in response to a wide range of air and soil-brone pollutants and to herbicides (Bradshaw& McNelly, 1981; MacNair, 1981; Murphy, 1983; Taylor,1990)

1.3.1 Different types of contamination in plants

There are mainly two types of contamination present in plants and foods.

Biological (ants, flies, fungi, bacteria, etc.) and chemical (petrol, fertilizer, soaps, cleaners, detergents etc.). Our main concerned was on chemical contaminants, which can be air-borne, water-borne and soil-borne.

1.3.1a Air-borne contamination

Diesel and petrol fuelled vehicles are responsible for the generation of a wide range of pollutants, with concentrations and relative proportions of pollutants depending on vehicle technology and operating conditions [91]. In terms of their effects on plants and their relatively high concentrations in exhaust emissions, nitric oxide (NO) and nitrogen dioxide (NO_2) are the most important phytotoxic pollutants associated with road transport [90]. However, trace amounts of other nitrogen-containing compounds such as nitrous acid (HONO), nitrous oxide (N_2O) and ammonia (NH_3) may also be present in vehicle emissions [89]. During combustion, other pollutants, including sulphur dioxide (SO_2) and volatile organic compounds (VOCs), are emitted, together with carbonaceous particles from incompletely burnt fuel droplets [91]. Concentrations of traditionally important pollutants such as sulphur dioxide (SO_2) and black smoke have declined substantially, whilst road traffic emissions have emerged as the major cause of poor air quality. Previous research has shown that at high concentrations, many of the pollutants present in exhaust gases can be damaging to plants [92,93].The interactions between plants and different types of pollutants were investigated by many authors: most studies on the influence of environmental pollution focus on physiological and ultrastructural aspects [94,95]. Studies concerning the anatomy of the vegetative organs under conditions of pollution have been also carried out [96]. Although fumigation experiments with exhaust gases under controlled conditions have tended to confirm the findings from field studies [90].

1.3.1b water-borne contamination

The problem associated with the disposal of sewage water and municipal wastes are important aspects of water pollution which should be considered in the context of wider environmental problem. Application of waste water for Irrigation purposes has increased over the past years. This waste water also contains high amounts of trace elements and heavy metals. Waste waters are contaminated with trace elements like Lead (Pb), Cadmium (Cd), Nickel (Ni), Mercury (Hg), Copper (Cu), Zinc (Zn), Boron (B) , Cobalt (Co), Chromium (Cr), Arsenic (As), Molybdenum (Mo), Manganese (Mn), etc. Many of these are non- essential and are toxic to plants, animals and human beings. Sensitivity of vegetable crops like lettuce, parsley, cabbage,

onion, spinach, carrot, radish, tomato etc. to water pollutant s has been reported. Waste water is suitable for irrigation if the content of toxic elements is reduced considerably. Long term intake of leafy vegetables grown on sludge constitutes a possible health hazard to consumers [97].Concentration of Pb was high in spinach, cabbage and spinach. Spinach had higher concentration of Cd when grown on urban sewerage sludge. Concentration of Mn was found to be 60% higher in sewerage treated fields [98]. Concentration of Zn, Cu, Mn, Ni, and Cd were higher in tomato and cabbage plants grown in high metal media than grown on low metal media [105]. Cd, Pb and Zn concentration was high in potato grown in sewerage sludge [103].

Toxicity of as many as 76 priority pollutants was observed in lettuce present in soil and nutrient solutions [100].Uptake of Cd, Cr, Pb and Zn increased in lettuce as the dosage of sludge was increased [102]. Contents of heavy metals particularly Zn in plants of spinach, fenugreek and lettuce increased at increasing rate of sewage sludge [101]. Cauliflower curds tend to accumulate high amounts of Zn, Cu, Fe, Mn, Pb and Ni than leaves when grown on sewage sludge [99]. As Cr concentration in soil solution increases, Cr uptake and translocation is also increased [104].

1.3.1c Soil-borne contamination

Extensive industrial production is usually connected with the emission of various pollutants to the environment. Among the most dangerous are heavy metals which accumulate in the ground, soil and bottom sediments of seas and oceans over long periods of time and effect biotic factors of the environment. Heavy metal contamination of soil affects the quality of crops, which very often reduces and sometimes disables the production of valuable food and animal feed [107]. Plants harvested from heavy metal polluted areas are usually tested for heavy metal concentration while the concentration of other elements is often neglected. Plants can easily uptake cadmium and transfer it to other organs [107].

The excessive intake of these elements from the soil creates dual problems; first the harvested crops get contaminated, which serve as a source of heavy metal in our diet, and secondly the crop yield decline due to the inhibition of metabolic processes [110] Zinc, copper, iron, manganese, lead and cadmium contents in soils receiving sewage sludge accumulated mostly in the top 0 to 15 cm layers. Heavy metals occur in the soil both in soluble and combined forms. However, only soluble exchangeable and chelated metal species in the soils are mobile and hence available to the plants [111]. Metal accumulation in plant depends on plant species, growth stages, types of soil and metals, soil conditions, weather and environment [108]. Thus accumulation of heavy metals in the edible parts of vegetables represents a direct pathway for their incorporation into the human food chain [109].

1.3.2 Bioaccumulation

As we discussed above the elements in plants are mainly taken up by root and above ground tissues from the underneath soil due to bioaccumulation. The levels of bio accumulated metals in the plants depend on the concentration of metals in soil and nature of the metals, whether it is non-biodegradable or non-thermo degradable and transportation of metal from one system to another in a food chain. Thus vegetable plants may acts as a medium for transferring higher levels of heavy metals from contaminated soil to food chain and causes metal toxicity and related human disease [112, 113]. The literature reports showing elevated levels of heavy metals in edible part of vegetables and plants. Plants take up both essential metal (Cu, Cr, Fe, Cu, Zn, and Se) and non-essential (As, Cd, Hg, and Pb) metals non-specifically. The biological activities related to many of these metal ions have been discussed by [114].

1.4 Inorganic elements: Their biological importance

The inorganic elements in biological systems can be classified in to three categories:

Structural elements: C, H, O, N, P, S and Ca.

Macronutrients (elements acts as electrolyte found in mg g^{-1} levels): Na, K, Mg, Cl, and Ca.

Trace elements: classified as essential and non-essential elements.

Certain elements present in body in μg g^{-1} levels and called as micronutrients. The important functions of these macro and micronutrients have been elaborately discussed by Fraga *et al.* 2005. Essential elements play important roles in several biochemical processes. They are: Co, Cr, Cu, Fe, I, Mn, Mo, Zn, Se, Ni, F, and V.

The physiological effects of essential elements depend on the level of intake or absorption in the body. The optimum range of these elements provides proper functioning to human system. If intake is less than the optimum range, than functioning will be improper and deficiency syndrome will occur. On the other hand, toxic effects start appearing when the intake exceeds the safe limit. The major functions and deficiency symptoms which led to the recognition of essential mineral elements has been illustrated in Table 1.1 (Annexure-II).

In addition, certain elements are non-essential elements and referred to as toxic elements, namely: As, Br, Cd, Hg, Pb, Se, Sb, Tl. The toxicity of an element was found to depend on the oxidation state, as outlined in Table 1.2 [115].

1.5 Plant based food materials

As part of any healthy eating plan, it is particularly important to include mainly plant-based foods, such as cereals, grains, rice, pasta, bread, vegetables, legumes and fruits. These are low in saturated fat, high in vitamins and minerals, and contain many other components that help to fight diseases such as heart disease and cancer. Plant-based foods are high in fibre, which reduce constipation [www.heart foundation.org., 116].

Many plants or plant parts are eaten as food. Seeds of plants are a good source of food for animals, including humans, because they contain the nutrients necessary for the plant's initial growth, including many healthful fats, such as Omega fats. In fact, the majority of food consumed by human beings is seed-based foods. Edible seeds include cereals (maize, wheat, rice, et cetera), legumes (beans, peas, lentils, et cetera), and nuts. Oilseeds are often pressed to produce rich oils - sunflower, flaxseed, rapeseed (including canola oil), and sesame. Seeds are typically high in unsaturated fats and, in moderation, are considered a health food, although not all seeds are edible. Large seeds, such as those from a lemon, pose a choking hazard, while seeds from apples and cherries contain a poison (cyanide).

Fruits are the ripened ovaries of plants, including the seeds within. Many plants have evolved fruits that are attractive as a food source to animals. Fruits, therefore, make up a significant part of the diets of most cultures. Some botanical fruits, such as tomatoes, pumpkins, and egg plants, are eaten as vegetables. Vegetables are a second type of plant matter that is commonly eaten as food. These include root vegetables (potatoes and carrots), leaf vegetables (spinach and lettuce), stem vegetables (bamboo shoots and asparagus), and inflorescence vegetables (globe artichokes and broccoli and other vegetables such as cabbage or cauliflower [en.wikipedia.org.,117].

1.5.1 Quality of plants based food materials

Research shows that plant-based foods are superior in nutrition to foods that are derived from animal sources. Studies confirm that both the quantity and the quality of nutrients in plant-based food far exceeds that of the nutrients obtained from animal sources. Fueling your body with plant-based foods also provides optimal protection from chronic diseases, such as heart disease and colon cancer.

Carbohydrates, regarded by many as energy food, are available in three varieties: starch, sugar, and fiber. Grains, a kind of starch, are often consumed to increase energy levels during exercise. It is becoming widely accepted that whole grains have greater nutritional value than their milled, processed, and refined counterparts, i.e. flour, sugar, and pre-cooked rice. In its entirety, the grain contains a plethora of micronutrients that are stripped away during processing.

Once these essential nutrients have been eliminated, the food is virtually nothing but empty calories. It is commonly believed that in order to get adequate dietary protein for building strong muscles and bones, you need to consume meat and dairy products. The reality is that per calorie, plant-foods supply far more protein and other nutrients to the body than animal foods do, providing no cholesterol, very little saturated fat, and no animal protein which has been linked to cancer, heart disease, diabetes, and a host of other chronic illnesses.

Plants, whose chemical content has been shaped by thousands of years of evolution, produce two types of metabolites;

Primary metabolites: protein, lipids, carbohydrates, vitamins, fibres, minerals; the resulting products are generally intended with a nutrirtional aim and their use raises no serious problem.

Secondary metabolites: alkaloids, polyphenols, carotenoids, coumarins, quinones, terpenes, lignanes etc; medicines containing them have been marketed and they are currently being increasingly used for physiological purposes; most such plants, which border on those used in herbal medicine, represent a genuine problem in that legislation concerning them in the area of plant-based food supplements varies widely from country to country or may even be non-existent. So special attention will be paid to the secondary metabolites of plants [guide lines are given by partial Agreement, in The Social and Public Health Field, 2005, 118]. But nowadays computer vision technology is used for food quality assurance.

1.5.2 Metabolites to be analyzed

As stated above mostly secondary metabolites are required to be analysed.

Phenolics are broadly distributed in the plant kingdom and are the most abundant secondary metabolites of plants. Plants polyphenols have drawn increasing attention due to their potent antioxidant properties and their marked effects in the prevention of various oxidative stress associated diseases such as cancer. The identification and development of phenolic compounds or extract from different plants has become a major area of health and medical-related research. The mechanism of action involving antioxidant and pro-oxidant activity as well as interference with cellular functions is discussed [119].

Plant phenolics include phenolics acids, flavonoids, tannins (Figure 2) and the less common stilbenes and lignans (Figure 3). Flavonoids are the most abundant polyphenols in our diets. The basic flavonoid structure is the flavan nucleus, containing 15 carbon atoms arranged in three rings (C6-C3-C6), which are labeled as A, B and C. Flavonoid are themselves divided into six subgroups: flavones, flavonols, flavanols, flavanones, isoflavones, and anthocyanins, according to the oxidation state of the central C ring. Their structural variation in each subgroup is partly due to the degree and pattern of hydroxylation, methoxylation, prenylation, or

glycosylation. Some of the most common flavonoids include quercetin, a flavonol abundant in onion, broccoli, and apple; catechin, a flavanol found in tea and several fruits; naringenin, the main flavanone in grapefruit; cyaniding glycoside, an anthocyanin abundant in berry fruits (black currant, raspberry, blackberry, etc.).

Phenolic acids can be dived into two classes: derivatives of benzoic acid such as gallic acid, and derivatives of cinnamic acid such as coumaric, caffeic and ferulic acid. Caffeic acid is the most abundant phenolic acid in many fruits and vegetables, most often esterified with quinic acid as in chlorogenic acid, which is the major phenolic compound in coffee. Anther common phenolic acid is ferulic acid, which is present in cereals and is esterified to hemicelluloses in the cell wall [120].

1.5.3 Methods used for analysis

Antioxidants, neutralize free radicals or their action [85][molecules]. They act as the levels of prevention, interception and repair. Preventive antioxidants prevent the formation of reactive oxygen species (ROS). These include superoxide dismutase (SOD), which catalyses the dismutation of superoxide to H_2O_2 and catalase are those which break down hydrogen peroxides to water[99]. Interception of free radicals is mainly carried out by scavenging oxygen centred radicals (e.g., peroxyl radicals). These are promoted by some non-enzymatic antioxidants, namely, vitamin C (ascorbic acid), vitamin E (α-Tocopherol), glutathione, thiol compounds, carotenoids, polyphenols, flavonoids, etc. Certain enzymatic antioxidants are available for repair and reconstruction of injured cells [87-89]. Presence of free radicals cause oxidative stress related disease in our body. Infact, pro-oxidants in the form of reactive oxygen and nitrogen species (ROS and RNS respectively) are naturally produced in our body during various metabolic processes, but they are kept within the control levels by the built-in antioxidant defence [119].

One of the significant actions of antioxidants is to inhibit lipid peroxidation. Lipid peroxidation is a free radical mediated process as given below:

$$LH + {}^{\bullet}OH \longrightarrow L^{\bullet} + H_2O$$

$$L^{\bullet} + O_2 \longrightarrow LOO^{\bullet}$$

$$LOO^{\bullet} + LH \longrightarrow L^{\bullet} + LOOH$$

$$LOO^{\bullet} + \alpha\text{-}TOH \longrightarrow LOOH + \alpha\text{-}TO^{\bullet}$$

When free radical (•OH) attacks a lipid (LH), it can abstract a hydrogen atom from a methylene group (CH_2) leaving behind an unpaired electron on carbon on carbon atom (•CH). This carbon radical is stabilized by molecular rearrangement to produce a conjugated diene, which can then react with an oxygen molecule to give a lipid peroxyl radical (LOO•). These radicals can further abstract hydrogen atoms from other lipid molecules to form lipid hydroperoxide (LOOH) and at the same time propagate LP further. The peroxidation can be terminated by a number of reactions. The most significant one involves reaction of LOO• or lipid radical (L•) with a molecule of antioxidant such as vitamin E or α-Tocopherol (α-TOH), which forms a more stable tocopherol phenoxyl radical, which is not involved in further chain reaction. The process of LP give rise to many products of toxicological interest, like malondialdehyde (MDA), 4-hydroxynonenal (4-HNE) and various 2-alkenals. The schematic representation of MDA formation is given in fig. 1.2. Devasagayam et al. [87] has briefly reviewed the possible interaction of free radicals with carbohydrate, DNA and proteins.

The antioxidant property is conventionally measured as total phenolic contents and total flavonoid contents. The antioxidant activities are assayed by measuring the extents of inhibition of a commercially available free radical ($DPPH^*$) by increasing the concentration of plants extracts. Single analysis has not been accurate for assessing antioxidant activities of plant based food materials. A series of antioxidant activities, namely, metal chelating ability ferric reducing power, and lipid peroxidation inhibition are done to assess the quality of a medicinal plant as a sources of antioxidants.

The inorganic elements are in the range of $\mu g\ g^{-1}$, so it demands low detection limits. Apart from the detection limit, we should also take care of other parameters, like multielemental capability, accuracy, precision, sample preparation method, and analysis time, size of sample and reproducibility and minimum interference from sample matrix for elemental analysis techniques.

The analytical techniques suited for performing trace element analysis are:

Inductive coupled plasma (ICP) atomic emission spectroscopy or mass spectroscopy as detector (ICP-AES and ICP-MS respectively)

Atomic absorption spectroscopy (AAS)

Flame photometry (FP)

X-ray fluorescence spectrometry (XRF)

Particle induced X-ray emission (PIXE)

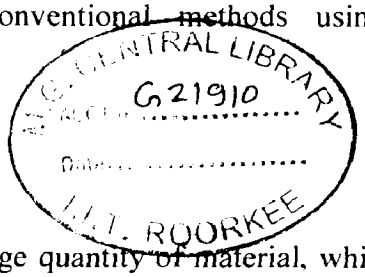
Neutron activation analysis (NAA)

Among these techniques, the most used is AAS, FM, and ICP-MS. While techniques like XRF and PIXE have not been very popular. Still NAA remains the only technique, which is non-destructive, with multielemental capabilities, needs minimum sample preparation, and is not affected by complex sample matrix.

AAS and ICP-MS are becoming popular for determing metals and metalloids in biological and environmental samples in solution form. AAS is based on the principle of absorption of monochromatic light to measure the concentration of an element in gas-phase. It is primarily single element analytical technique, though multielemental hollow cathode lamps are available. Both ICP-MS and AAS are destructive techniques as the sample has to be in solution phase, which is aspirated into flame and the quantification is based on optical measurement. In ICP-MS, the plasma technology and the temperature involved improves the excitation process and the sensitivity further increased by replacing optical detection method AES by MS, where the ions of the elements to be detected are directly measured. It is multielemental in nature where different ions are separated using the concept of variation in bending ions of different masses by applying magnetic field. The detection limit for ICP-MS are order of the ng/g(ppb) or less and are finding applications in different fields, like environmental science, food chemistry, geology, biology , etc.

On the other hand, XRF and PIXE based on measuring characteristic X-rays by exciting the samples with X-ray source and protons respectively. The detection limits for these techniques are poorer as compared to AAS and ICP-MS.

It's a great challenge for us to quantitative analyze vegetable plants due to their complex matrix character. These vegetables are grown in natural environment, so they are susceptible to contaminations that prevail locally. We have used Inductively Coupled Plasma Mass Spectrometry (ICP-MS), Atomic Absorption Spectrometry and Flame Photometry (FP) for measuring the concentrations of the trace and minor elements in vegetable plants. Antioxidant activities were determined by the following conventional methods using UV-Visible spectrophotometric measurements.



2.1 Sampling

It is a process of extracting an aliquot from a large quantity of material, which should be a true representative of whole material to be analyzed. Sample collection and storage is an important part of sampling. It is known that the plant materials of same species but grown in different soil, climate and other environmental condition, show significant variation in the characteristics. Different parts of the same plant, namely, root, stem, leaves, bark, fruit, and seeds have elemental compositions are also different. In this study we have chosen our study area in the vicinity of active copper mine to evaluate the effect of mining activites on the qualities of vegetable plants. We choose Khetri region in Rajasthan which is the largest active copper mine in India. About four common vegetable plant species with local names given, which are chosen for studies namely, *Spinacia oleracea* (palak), *Brassica oleracea* (gobhi), *Trigonella foenum-graecum* (methi), and *Brassica juncea* (sarson) were taken from the periphery of the mining area and collected from eight to ten locations within 20 km^2. This mining area will be named as mining impact site. Similar vegetable species were collected from non-mining area which will name as control site. We have chosen Haridwar as control site which is a part of fertile Ganges Basin and is used for cultivation of numerous vegetable plants.

All plants sample collected were sealed in pre-cleaned, pre-weighted zip-lock polythene bags and transported to our laboratory. Plants samples were weighted and then washed with tap water rinsed with Milli-Q water to remove surface dust particles and extraneous materials [121] and were kept for drying to remove moisture content. Samples are air-dried in sunlight and then in an oven at 70-80 ^{o}C for 48h. After withdrawing the samples from oven, it was kept in a desiccators so that moisture does not get reabsorbed. For safe side we use laminar hood and a dust free chamber for sample handling and sample preparation.

Sample homogenization was done by crushing, grinding and pulverizing the dried sample in agate mortar. Samples were then sieved to uniform particle size by passing through 100 mesh size sieve. In some report mentioned that sample size is necessary of analysis [122]. The sample preservation and storage is to prevent any undesirable change between the time sample is collected and analysis is done. The matrix properties, humidity, *p*H, temperature, duration of storage and container material are linked to the contamination and to maintain originality of the

sample. Samples are recommended to be stored at -4°C. Soxhlet extraction was done of these plants samples with MeOH Fig. 2.1 (Annexure –I). 5gm of dried powdered sample was extracted in 200mLof MeOH for 8 h at 40-60°C. Then this extracted solution should be evaporated by Rota evaporator, and then lyophilizations was done to get vegetable plant extract in dried from and then weight the dry extract of plant sample. Dry extract of plant is used to calculate the % extraction.

% extraction = [Dry plant extract/wt. of dry powdered plant] X100

2.2 Chemical reagents

For elemental quantitative analysis we need 97-98 % HNO_3, 97% $HClO_4$, 98-99% H_2SO_4, 30% H_2O_2, and Millipore-Q water. For antioxidant assay, 2,2'-Diphenyl-1-picrylhydrazyl free radical (DPPH•), 3-(2-pyridyl)-5,6-bis(4-phenyl-sulphonic acid)-1,2,4-triazine (ferrozine), (+)-catechin, oleic acid ferrous chloride were obtained from Sigma-Aldrich GmbH, Germany. Folin-Ciocalteu reagent (F-CR), gallic acid, aluminum chloride, methanol, trichloroacetic acid (TCA), Oleic acid, sodium nitrite, hydrogen peroxide, Thiobarbatiuric acid (TBA) and sodium carbonate were obtained from Merck India. Butylated hydroxyltoluene (BHT) was purchased from Himedia, India. All the chemicals were used without further purification.

2.3 Trace Element Analytical techniques

There are different techniques for trace element analysis. Each technique has its own advantage and disadvantage. We mainly used Inductive Coupled Plasma Mass Spectrometry (ICP-MS) and atomic spectrometry (AAS) for analyzing elements. We use Flame Photometer to analysis some alkali metal.

2.3.1 Atomic Absorption Spectrometry (AAS)

This technique is used to analyze trace elements. It consist of 4 basic structural components; a light source (hollow cathode lamp), an atomizer section for selecting the wavelength of the target element, and a detector for converting the light into electrical signal. It utilizes the phenomenon that "free" atoms in gaseous state absorb radiation at specific wavelength. Actually, sample is in liquid state and the element in the sample to be analyzed by AAS is usually in bounded form. The element is converted in to free atom in vapor state by apply heat energy, either by flame (flame atomization) or by electric furnace (electro thermal atomization). The measured absorbance by free metal atoms is correlated to their concentration and follows Beer Lambert's Law [123].

The inorganic content of most vegetable plants is only a minor constituent and interfered by major constituents. So, we first need to ensure that the analyte of interest is released from sample matrix, by decomposing organic matter. Many decomposition methods are available for determination of trace elements in plants, including dry ashing and wet digestion. Besides this microwave digestion is also frequently used in AAS as described by Akman *et al.* [124] for trace heavy metal contents in cereals, pulses and spices.

2.3.1.1 Instrumentation

The element analyses were carried out using the atomic absorption spectrophotometer at Department of Chemistry IIT Roorkee. It uses acetylene-air/acetylene-nitrous oxide mixture to ignite flame. The operational conditions including the adjustment of flame height are software controlled. Hollow cathode lamps of the respective elements were used. For each element the instrument was first calibrated using at least four standard solutions of the element which were obtained from the Merck Specialities Private LTD Mumbai (India). The flow rate of air and acetylene was maintained at 10.1 and 2.6 L/min respectively. The calibration curves for the element of interest (given as supporting data in Annexure-I) were obtained by measuring standard solutions of the elements (Merck) in the working range as mentioned in the Table 1.3 (given in Annexure-II). The standard solutions were prepared by serial dilution of stock solution of 1000mg/L of the respective element.

2.3.1.2 Sample Preparation

An accurately weighed ~2.0g of dried powdered plant samples were digested carefully with 5:1 mixture of nitric acid (25%v/v) and perchloric acid, followed by controlled heating until the evolution of gases ceased. A 15 ml of deionized water (Milli-Q, resistivity of 18.2 $M\Omega\ cm^{-1}$) was added to this mixture and filtered through a 0.45 μm Millipore membrane into a 25 mL volumetric flask. The membrane containing small quantity of residue was washed with deionized water to make up the volume.

2.3.2 Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

It is highly sensitive technique for element detection, where inductively coupled plasma is used to produce ions and mass spectrometry is used for detection of ions. The produce during the process are separated by magnetically. It is capable of the determining a wide range of metals and non-metals at concentration at ng g^{-1} (ppb). The plasma technology is used for achieving high temperature which is used for ionization of the samples, so singly charged ions are formed (most cases).this technique is more précised and sensitive as compared to AAS.

2.3.2.1 Instrumentation

We have used ICP-MS (Perkin Elmer Sciex make) with argon plasma as the excitation source (Institute Instrumentation Centre, Indian Institute of Technology, Roorkee). The instrument is equipped with ELAN® DRC-e facility which uses a technology to chemically remove interferences from the ion beam before they enter the mass analyzer quadrupole. This method offers very good detection limit of the order of ng g^{-1} or ppb due to low background levels as the matrix effect is lowered significantly at high plasma temperature as compared to atomic absorption method. The detection limit is further improved by using non-optical method, namely, mass spectrometer.

The calibration curves for the elements of interest (given as supporting data in Annexure-I) were obtained by measuring standard solutions of the elements (as used as making AAS standard, Merck Specialities Private LTD Mumbai, India) in the recommended working range. The standard solution was prepared by serial dilution of 1000ppm stock solution of the respective element. The ICP-MS operating condition (Table 1.4) was optimized using built-in software.

The accuracy and precision of the ICP-MS method validated by triplicate analysis of NIST apple leaves reference materials (Table 3.1). A blank, prepared with the reagents used for sample preparation for ICP-MS measurement, was analyzed with each batch and all data was reported in metal concentrations (μg g^{-1} dry weight).

2.3.2.2 Sample preparation

Accurately weighed 1 g of each powdered plant sample was acid digested according to the method given by Wang *et al.* [125]. Powdered samples were digested with 10mL of concentrated HNO_3 at 95°C for an hour. Then add 5ml H_2SO_4 at 140°C followed by 5mL concentrated HNO_3 at 180°C. Further add 1mL of H_2O_2 and heat until brown fumes ceased to appear and turned into a colorless solution and was in semi-dry condition. Then the digest was cooled down. A 10mL of distilled water and 0.5mL of HNO_3 was added at 200°C until white fumes appear and filtered through o.45 μm Millipore membrane in 50mLvolumetric flask, and then made up to the mark with deionized water for analysis to total contents of Cu, Cd, Fe, Mn, Zn, Ni, Pb, and Cr by ICP-MS.

2.3.3 Flame photometer

Flame photometry (more accurately called flame atomic emission spectrometry) is a branch of atomic spectroscopy in which the species examined in the spectrometer are in the form of atoms. The atoms under investigation are excited by light. Absorption techniques measure the

absorbance of light due to the electrons going to a higher energy level. Emission techniques measure the intensity of light that is emitted as electrons return to the lower energy levels. Flame photometry is suitable for qualitative and quantitative determination of several cations, especially for metals that are easily excited to higher energy levels at a relatively low flame temperature (mainly Na, K, Rb, Cs, Ca, Ba, and Cu).

This technique uses a flame that evaporates the solvent and also sublimates and atomizes the metal and then excites a valence electron to an upper energy state. Light is emitted at characteristic wavelengths for each metal as the electron returns to the ground state that makes qualitative determination possible. Flame photometers use optical filters to monitor for the selected emission wavelength produced by the analyze species. Comparison of emission intensities of unknowns to either that of standard solutions (plotting calibration curve), or to those of an internal standard (standard addition method), allows quantitative analysis of the analyze metal in the sample solution.

2.3.3.1 Instrumentation

The flame photometers are relatively simply instruments. We have used ELICO (CL 378) flame photometer in Department of Chemistry, Indian Institute of Technology, Roorkee (fig.2.2) . In flame photometer there is no need for source of light, since it is the measured constituent of the sample that is emitting the light. The energy that is needed for the excitation is provided by the temperature of the flame (2000-3000 °C), produced by the burning of acetylene or natural gas (or propane-butane gas) in the presence of air or oxygen. By the heat of the flame and the effect of the reducing gas (fuel), molecules and ions of the sample species are decomposed and reduced to give atoms. Atoms in the vapour state give line spectra. The most sensitive parts of the instrument are the aspirator and the burner. The gases play an important role in the aspiration and while making the aerosol. The air sucks up the sample and passes it into the aspirator, where the bigger drops condense and could be eliminated. The monochromator selects the suitable (characteristic) wavelength of the emitted light. The usual optical filters could be used. The emitted light reaches the detector. This is a photomultiplier producing an electric signal proportional to the intensity of emitted light.

2.3.3.2 Sample preparation

Sample preparation for flame photometry is similar as sample preparation done for AAS (2.3.1.2).

2.3.3.4 Bioaccumulation in vegetable samples

To determine the heavy metals levels and bioaccumulation factors in vegetable plants grown in two different environmental conditions namely, mining impact site where soil is metalliferous and control site where soil is not contaminated with heavy metals. The concentration of heavy metals in vegetable plant sample is in the range of $\mu g\ g^{-1}$(ppm) and can be measured by ICP-MS and AAS.

The sample preparation was similar to AAS (2.3.1.2).

2.4 Antioxidant Activities of Vegetable Plants

In order to assay the antioxidant activities of the vegetable plant sample, it is essential to first extract the relevant compounds in a suitable solvent. We use methanol as a solvent to study the antioxidant activities for their respective extracts.

2.4.1 Preparation of plant extracts (Methanol extract)

5 g of powdered vegetable plant of individual sample were extracted successively with 200mL methanol in a soxhlet apparatus for 8h as shown in fig. 2.1 (Annexure- I). The extracts were filtered and evaporated till dryness under reduced pressure and control temperature (40°C) in a rotary evaporator and then lyophilized to obtain freeze dried crude extract, which were stored at -20°C till further use. For assaying purpose, the dried extract was dissolved in MeOH.

2.4.2 Analysis of antioxidant activities in vegetable plants

2.4.2.1 Estimation of total phenolic contents (TPCs)

The TPCs in the crude extract of the vegetable plant samples from mining and control sites determined from calibration curves, which are obtained using known quantities of standard antioxidants. The TPC is determined according to the Folin-Ciocalteau method [126] using Gallic acid as the standard. In this method 100µL of F-C reagent is mixed with an aliquot of 320µL of plant extract followed by 1.58mL distilled water. After 8 min incubation add 300µL of 20%(w/v) Na_2CO_3 and then the mixture is allowed to stand for 2 h at 25°C in dark, the absorbance of the solution is measured at λ_{max} = 760nm and compare with Gallic acid calibration curve (y= 1.23673x+0.02171; R^2 =0.9989; y is the absorbance at 760nm and 'x' is the concentration of standard antioxidant) shown in fig. 3.1 and 3.2 (Annexure-I). The total phenolic content is determined as Gallic acid equivalents (GAE in mg/weight of the extract in g), and the values are presented as average of triplicate analyses.

2.4.2.2 Estimation of total flavonoid contents (TFCs)

The TFCs in the crude extracts of the vegetable plant samples from mining and control sites are determined by treating 0.25mL of the extract with 1.25mL distilled water and 75µL of 5%(w/v) $NaNO_2$ solution and maintained for 6 min [261]. Similar process is used for assaying (+)- catechin as standard. Then 0.15mL of 10% $AlCl_3.6H_2O$ solution was added and after 5 min, 0.5 mL of 1M NaOH was added. The mixture was diluted to 2.5mL with double distilled water and its absorbance was measured immediately at λ_{max} = 510 nm, which was compared with the standard curve of (+)- catechin (y= 1.20801x+ 0.0307; R^2 =0.99807; y is the measured absorbance and 'x' is the concentration of the solution) shown in fig. 3.4. The TFC was determined as catechin equivalents (CEs in mg/weight of the plant extract in g), and the values are represented as average of triplicate analyses.

2.4.2.3 Determination of DPPH• radical scavenging activity

Substances capable of scavenging free-radicals by donating hydrogen or an electron to it, can be considered as antioxidants. 2,2'-diphenyl-1-picrylhydrazyl (DPPH•) is one of the few stable and commercially available free-radical, which is violet in color (λ_{max} = 517nm). In the presence of different doses of vegetable plants containing antioxidants, the intensities of violet color of DPPH• free radical are reduce to 2,2'-dephenyl-1-picrylhydrazine, which is yellow in color. The mechanism is shown in fig. 2.3. The degree of discoloration of violet color of DPPH• free radical indicates the radical scavenging potential, and decrease in the absorbance is directly proportional to radical scavenging activity [127].

The extracted samples of various concentrations e.g. 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000 µg/mL were prepared in methanol. 2mL of each extract solution was mixed with 2 mL of 0.2mM methanol solution of commercially available stable DPPH• radical. The mixture was incubated with vigorous shaking and allowed to stand for 30 min in dark condition. The absorbance of DPPH• radical at λ_{max} =517 nm decreased in the presence of antioxidants in vegetable plants. Rutin (RT) was used as reference standards. The activity to scavenge DPPH• radical was calculated using the relationship given by [128].

$$\text{Radical scavenging activity \%} = \left[\frac{A_o - A_s}{A_o}\right] \times 100$$

Where, I (%) is the percentage inhibition, A_0 is the absorbance of the control reaction containing all reagents except the plant extract, and A_1 is the absorbance of the plant extract. The corresponding IC_{50} values of DPPH• radical scavenging ability were determined.

2.4.2.4 Determination of chelating effects on ferrous ions

The Fe^{+2}nchelating ability of the vegetable plant extracts was measured by the ferrous ion-ferrozine complex method [129]. 2mL of various concentrations (100-1000 µg/mL) of the extracts in methanol was added to a solution of 2mM $FeCl_2$ (0.05mL). The reaction was imitated by the addition (0.2 mL) of ferrozine. Then, the mixture was vigorously shaken and left at room temperature for 10 min. spectrophotometric absorbance in sample solution A_1 was measured at λ_{max} = 562 nm. Absorbance of control (A_0) was determined by replacing the sample with methanol. EDTA solution (1- 1000 µg/mL) was used as a reference standard. The inhibition percentage of ferrozine-Fe^{+2} complex formations was calculated by using the given formula:

$$\text{Metal chelating activity (\%)} = \left[\frac{A_0 - A_1}{A_0}\right] \text{X } 100$$

2.4.2.5 Determination of Inhibition of Lipid peroxidation

The oxidative stress conditions, e.g., due to over production of peroxide free radicals, lipids could undergo oxidation leading to several oxidative degenerative disease. Supplementing antioxidants from external sources, *viz.* vegetable plants, would facilitate inhibition of lipid peroxidation process. This was estimated quantitatively by measuring the intensity of complex formed between reactive oxygen species (ROS) and thiobarbituric acid (TBA) to form TBRAS, according to the method given by [130]. The assay of lipid peroxidation reaction is schematically shown in fig 2.4 (Annexure-I) where, malondialdehyde is a product of oxidation of polyunsaturated fatty acid (e.g. oleic acid and linoleic acid).

The plant extract (10-500 µg/mL) was added to an aqueous solution containing 200µL of Tris-buffer (pH 1~7.4), 300µL of 1M KCl, 400µL of 1%SDS, 10µL of oleic acid. The oxidation was initiated by adding freshly prepared 40µL $FeCl_2$ (1.0 µM) and 20 µL of H_2O_2 (0.5 µM) solution in umber color vial. After incubation at 37°C for 16h, in dark condition, the reaction was stopped by cooling and adding 50 µL of 4% BHT in ethanolic solution and 2mL of 0.67%TBA solution, 20%TCA, 1% SDS and 5N HCl. The formation of the TBRAS adduct was achieved by heating the above mixture in water bath at 80°C for 60 min. and was cooled in the ice bath. The BHT was used as a standard antioxidant. Intensity of TBRAS was measures using a U.V-Visible

spectrophotometer at λ_{max} = 532nm. The percentage oleic acid peroxidation inhibition L (%) was calculated as:

$$L\ (\%) = \left[\frac{(A_o - A_1)}{A_o}\right] X\ 100$$

A_o = absorbance of control (without extract), A_1 = absorbance of extract, (blank was recorded without extract, $FeCl_2$ and H_2O_2 solution). The inhibition of lipid for plant extract of various concentrations was determined from the average of triplicate analysis. The IC_{50} values of inhibition of lipid peroxidation were determined.

All the antioxidant activities were measured using double beam UV-Visible spectrophotometer (**Shimadzu)**, with a wavelength scanning range of 190nm to 780nm.

3.1 Result and Discussion of Elemental Analysis

We have used three methods for elemental quantification in our vegetable samples.

3.1.1 Elemental Analysis of vegetable plants by AAS

Analysis of Cu, Fe, and Zn was done by AAS, measurement in triplicate by Air/Acetylene method. Here we used NIST Apple Leaves SRM-1515 as control samples. The certified values of NIST Apple Leaves SRM-1515 are shown in Table 3.1.

The comparison of measured elements concentration values (in $\mu g\ g^{-1}$) of Apple leaves reference material (NIST, SRM-1515) by ICP-MS and AAS are shown in table 3.2.

Mainly three main metals are measured in five vegetable plants from Mining Impact Site (shown in table 3.3 and 3.5) and two vegetable plants from Control Site (table 3.4 and 3.6). It was seen from the respective mean values and standard deviations that these vegetable plants have different uptake pattern for heavy metals.

3.1.2 Elemental Analysis of vegetable plants by ICP-MS

Analysis of Cu, Fe, Cr, Mn, Zn, Cd, Ni, and Pb was done by ICP-MS (Perkin Elmer Sciex). The details of the ICP-MS were already discussed in chapter 2. The calibration curves for the elements of interest (given in Annexure –I). The accuracy and precision of the ICP-MS method were validated by analyzing NIST Apple Leaves (SRM-1515) reference material. The quality control was done by analyzing the reference material after every ten samples and all the data was reported in metal concentrations ($\mu g\ g^{-1}$ dry weight). The certified values of Apple Leaves NIST, SRM-1515 is given in table 5.1. Here we relative method to determine the concentration of metals in vegetable samples. The measured elemental values present in vegetable samples from mining impact site and control site are given in table 3.3 and 3.4 respectively.

3.1.3 Alkali Metal Analysis of vegetable plants by Flame Photometry

Analysis of Na and K was done by Flame Photometry ELICO (CL 378) . As we know that alkali elements are the major elements present in any vegetable plants. Here also we get the high concentration of above mentioned alkali metals in our mining impact site and control site vegetable samples are given in table 3.7 and 3.8 respectively.

3.1.4 Bioaccumulation of metals in vegetable plants

To determine the BAFs in vegetable sample we need to know the elemental composition of soil of mining impact site and control site, which are shown in table 3.9 [131]. The bioaccumulation factor (BAFs) depends on the concentration of metal in plants leaves and in the soil.

The BAFs of Cr, Mn, Fe, Cu, Zn, Ni, Cd, and Pb in the plants samples from mining impact site and control site were calculated as

$$(BAFs)_{Mi} = \frac{[M_i]_{plants\ leaves}}{[M_i]_{soil}}$$

Where M_i is the i^{th} metal and $[M_i]$ is the concentration in $\mu g\ g^{-1}$ (dry mass) of the i^{th} metal. The BAFs of above mentioned metals in vegetable samples from mining impact site and control site was shown in table 3.10.

3.2 RESULT AND DISCUSSION

The concentration levels of major and minor elements in soil samples from Mining Impact Site and Control Site sampling sites are given in Table 3.9 [131]. It was noted that the Cu concentrations in Mining Impact Site soil were abnormally high (763 mg kg^{-1}), which is nearly 30 folds higher that the Cu levels in the soil samples collected from Control Site (26.4 mg kg^{-1}). The source of Cu contamination in the local soil could be attributed to nearby Cu mining activities. Such high Cu levels in soil are considered to be phytotoxic. In addition, the concentrations of Cr, Fe, Zn and Pb were found elevated in Mining Impact Site soil as compared to soil from Control Site. Furthermore, the Mining Impact soils were found to be rich in Mg, Al and Ca. The differences in the concentrations of Mn and Cd in the soil samples from both Mining Impact and Control were not statistically significant. the concentration levels of As, Pb,Ni, and Zn, could be considered within the range of normal levels for both Mining Impact Site as well as Control Site soil [132,133].

3.2.1 Heavy Metal in vegetable plants from Mining Impact Site and Control Site

Eight important heavy metals were measured in four vegetable plants collected from Mining Impact Site and two vegetable plants collected from Control Site. It was seen from the respective mean values and standard deviations(shown in table 3.3 and 3.4) that Fe level in all the vegetable plants collected from Mining Impact Site is ranged between (77.5-269.6 $\mu g\ g^{-1}$), which about 3 folds higher than the corresponding vegetable plants from Control site (74.9-104.8 $\mu g\ g^{-}$

^{1}). The accumulation of Cu levels in all vegetable plants from Mining Impact Site range between 25.3-5.3 μg g^{-1} (data taken by AAS; table3.5). This elevated level were about 2.5 fold higher than the corresponding vegetable plants from Control site 6.1-10.5 μg g^{-1}(table 3.6).

In addition, the Cr (0.2-0.4 μg g^{-1}) and Ni (0.6-1.2 μg g^{-1}) levels were also found to be nearly higher in Mining Impact Site vegetable plants as compared those from Control Site. However, Cd (0.02-15 μg g^{-1}) and Pb (0.05-0.18 μg g^{-1}) were found to be nearly 4 fold higher in Mining Impact Site samples those to Control Site. The concentration of essential elements namely Na (9389-1327.5 μg g^{-1}), K (2317-1510 μg g^{-1}) (Table 3.5 and 3.6 res.), Mn (40-68 μg g^{-1}), and Zn (21-32.1 μg g^{-1}) in the vegetable plants from Mining Impact Site were similar to those from Control Site and were well within daily dietary intake range.

The alkali metal concentrations in vegetable plant samples from both MIS and CS are shown in Table 3.7 and 3.8, conclude that these metal are in the range of daily dietary intake value.

3.2.2 Bioaccumulation Factors (BAFs)

The calculated BAFs of the heavy metals in vegetable plants from Mining Impact Site and Control Site are given in Table (by ICP-MS) 3.10. Among the heavy metals which were higher in concentration, namely, Cr, Cu and Cd in the vegetable plants from Mining Impact Site, the BAFs for Cr were lower in MIS samples, in particular for S. oleracea(CS) and T. foenum-graecum (CS) where BAFs for Cr were 2-folds and 4-folds lower compared to the respective CS samples and B. oleracea (MIS) have nearly same value as S. oleracea(MIS), similarly B.juncea (MIS) show BAF value nearly to T. foenum-graecum (MIS) . While, the BAFs of Cuin the MIS samples were ~32 folds lower in S. oleracea and ~22 folds lower in T. foenum-graecum as compared to the respective samples from CS, in addition B. oleracea (MIS) and B.juncea (MIS) have very less value. In both the cases, lower BAF for MIS samples indicated lower degree of accumulation of these heavy metals from soil in the plants though these heavy metals were higher in concentration in the Khetri soil. It implies a significant fraction of Cr and Cu in the Khetri soil is most likely to be present as immobilized species, unsuitable for plant uptake.

The BAFs of Cd in all the vegetable plants from MIS were higher than the CS samples but it is significantly high in T. foenum-graecum (CS). High BAFs of Cd in MIS was in accordance with the accepted values of Cd bioaccumulation [134] and may be attributed to the acidic nature of its soil (pH~6.3) and with decreasing pH, the solubility of cadmium increases. As a result the mobility of Cd ions increases, thereby facilitating bioaccumulation. Toxic levels of cadmium are known to induce oxidative stress in neuronal cells and cause neurodegenerative diseases [135]. Chronic intake of such high Cd in the form vegetable plants could potentially lead to Cd toxicity which might affect nervous system and cause neurological disorders. It was further noted that lower BAFs for other heavy metals were mostly found among the vegetable plants from MIS. Particularly, S. oleracea (MIS), and T. foenum-graecum (MIS) showed lower BAFs for Ni, Zn,

Pb, and Mn. The BAF value for Fe is higher in CS vegetable samples as compared to corresponding vegetable samples of MIS[136].

3.3 Antioxidant assay of vegetable plants

3.3.1 Total phenolic content

The antioxidants content in the MeOH extract of the powdered vegetable sample from mining site and impact site were determined as TPC (total phenolic content) given in table 3.11 and the standard gallic acid graph was shown in fig 3.1(Annexure-I). The results are given as mean and standard deviation, as calculated from triplicate analysis. The TPC of Cu mining impact site samples and control site, expressed as gallic acid equivalents (GAE), ranged from 12.82±1.84 to 22.63±0.22 mg/g dry extract for mining impact site; and 19.14±0.50 mg/g to 18.05±1.28 of dry extract for control samples.

3.3.2 Total flavonoid content

The antioxidant content in the MeOH extract of the powdered vegetable sample from mining site and impact site were determined as TFC(total flavonoid content) given in table 3.11 and the standard catechin graph was shown in fig 3.2 (Annexure-I).The results are given as mean and standard deviation, as calculated from triplicate analysis. The TFC, measured as (+)- catechin equivqlent (CE), ranged from 20.18±1.43 to 9.75±2.16 mg/g of dry extract for mining impact site; and 16.79±0.021 to 15.46±0.197 mg/g of dry extract for control samples.

3.3.3 DPPH free radical (DPPH•) scavenging activity

The antioxidant activities of the MIS samples and CS samples were evident from the increasing order of DPPH• scavenging activities as the concentrations of MeOH extracts of the samples were increased shown in fig. 3.3 and 3.4 (Annexure-I).

The corresponding IC_{50} values were determined, shown in tab.3.12 (Annexure-II), and inversely correlate with the TPC (R^2=0.998) and TFC (R^2= 0.99) for MIS and CS samples respectively. This indicate that phenolic compounds are more powerful scavengers of free radicals. The minimum IC_{50} value was observed in B. oleracea (NIS) 470 µg/mL and maximum IC_{50} value was observed for S. oleracea (CS) and T. foenum-graecum (NIS) nearly to 1000 µg/mL. T. foenum-graecum (CS), B.juncea (NIS), and S. oleracea (NIS) show average IC_{50} value range from 470 µg/mL to 660 µg/mL.

3.3.4 Metal ion chelating ability

The ferrous ion chelating ability by the MeOH extract of vegetable plants from both MIS and CS increased with the concentration of the plants extract shown in fig. 3.5 and 3.6 (Annexure-I).

The corresponding IC_{50} values were calculated, shown in tab.3.12 (Annexure-II). As DPPH assay, the minimum IC_{50} value is 670 µg/mL and maximum IC_{50} value is >1000 µg/mL for MIS samples. In the control sample the minimum IC_{50} value is 830 µg/mL and maximum is >1000 µg/mL. Although the IC_{50} values were lower for the MIS sample corresponding to their CS samples.

3.3.5 Inhibition of Lipid peroxidation (Anti lipid peroxidation)

The antioxidant activities of the vegetable plant sample from Cu MIS and CS were do not show any gradual increase of % inhibition of Lipid peroxidation. All the experimental work we done on inhibition of lipid peroxidation are showing very irrelevant data shown in fig. 3.7 (Annexure-I) for spinach.

The irrelevance nature of data may be due to Oleic acid which we used in our experimental procedure in place of Linoleic acid. All the reported literature describes the inhibition of Linoleic acid.

Conclusion

Vegetable plants grown in impact metalliferous soil constitute normal levels of essential metals required for several biological function in human body.

Potentially toxic elements like Cr, Cu, Cd, and Pb were present. These metals are considered as cumulative poisons. These metals cause environmental hazards and very toxic.

The concentrations of Cr, Cd, and Cu (not very much) were higher in MIS samples. The concentrations of Cd in MIS samples were at threshold level of daily dietary intake range.

The Na and K contents were found enriched in S. oleracea and T. foenum-graecum from MIS samples than corresponding samples from CS.

The bioaccumulation studies showed the transport of heavy metals from one system (soil) to another system (vegetable plants) which threatens to cause toxicity as these heavy metals in vegetable plants are liable to enter into human food chain system.

The BAFs shows high accumulation of Cu metal in CS vegetable plant sample than the MIS vegetable samples, this is because the Cu at MIS is in non-exchangeable from (chemically bonded) while in CS Cu is in free from so that it accumulate more than in the corresponding MIS vegetable plant samples.

The concentrations of Fe, Mn, Zn, metal are in the range of the daily dietary intake.

The antioxidant activity was measured by several assay, B. oleracea from the MIS shows highest phenolic content which are mainly responsible for radical scavenging as it have highest value of TPC.

TFC value is maximum in S. oleracea (MIS), shows high level of flavonoid content in it.

The maximum DPPH radical scavenging activity was shown by B. oleracea (MIS) because of high phenolic content. Similarly it also show high metal chelating activity due to highest value of TPC.

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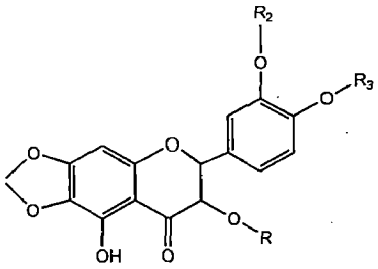
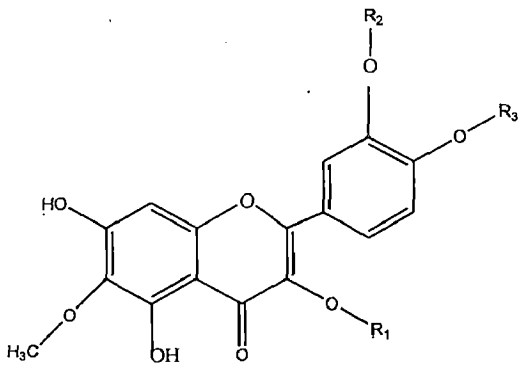
Annexure -I

Figures



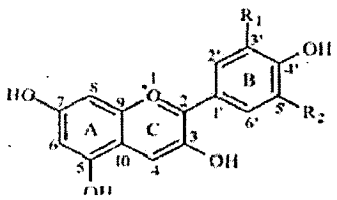
Compound 1: R1 = CH3; R2 = H; R3 = Glucuronic acid (Aritomi and Kawasaki [137]; Aritomi et al [138]) OR R1 = Glucuronic acid; R2 = H; R3= CH3 (Edenharder et al[139])



Compound 1: R1 = β-D-glucopyranosyl (1 6)-[β-D-apiofuranosyl (1-2)]-β-D-glucopyranoside; R2 & R3 = H
Compound 2: R1 = β-D-glucopyranosyl (1 6)-β-D-glucopyranoside; R2 & R3= H
Compound 3: R1 = β-D-glucopyranosyl (1 6)-[β-D-apiofuranosyl (1-2)]-β-D-glucopyranoside; R2= CH3; R3 = H

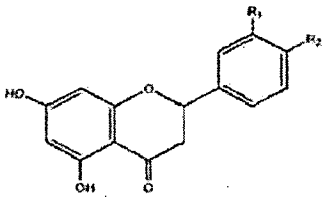
Fig. 1.1 Structures of the four major flavonoids in spinach

Anthocyanidin



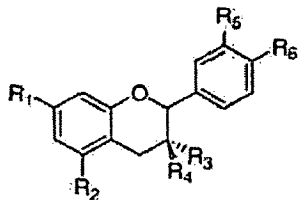
Delphinidin: $R_1 = R_2 = OH$
Cyanidin: $R_1 = OH$, $R_2 = H$

Flavanone



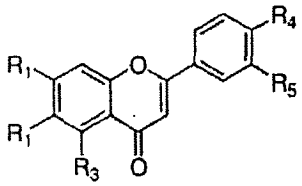
Hesperetin: $R_1 = OH$, $R_2 = OCH_3$
Naringenin: $R_1 = H$, $R_2 = OH$

Flavanol



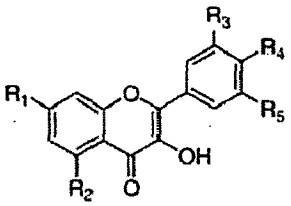
Catechin: $R_1 = R_2 = R_4 = R_5 = R_6 = OH$, $R_3 = H$
Epicatechin: $R_1 = R_2 = R_3 = R_5 = R_6 = OH$, $R_4 = H$

Flavone



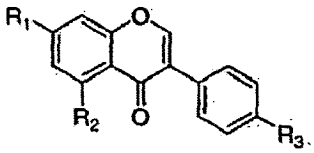
Apigenin: $R_1 = R_2 = R_4 = OH$, $R_2 = R_5 = H$
Luteonin: $R_1 = R_2 = R_4 = R_5 = OH$, $R_2 = H$

Flavonol



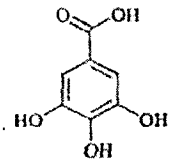
Quercetin: $R_1 = R_2 = R_3 = R_4 = OH$, $R_5 = H$
Myricetin: $R_1 = R_2 = R_3 = R_4 = R_5 = OH$

Isoflavone



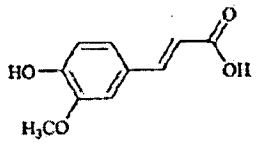
Genistein: $R_1 = R_2 = R_3 = OH$
Daidzein: $R_1 = R_3 = OH$, $R_2 = H$

Hydroxybenzoic acid

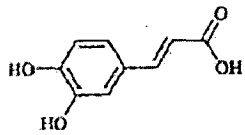


Gallic acid

Hydroxycinnamic Acid



Ferulic acid



Caffeic acid

Fig.1. 2. Structures of flavonoids and phenolic acids

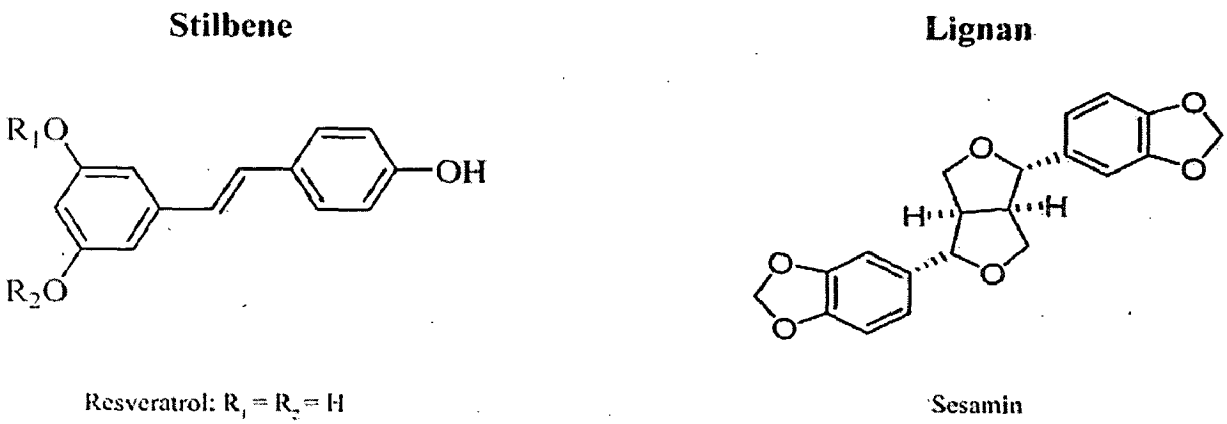


Fig. 1.2. structures of stillbenes and lignans

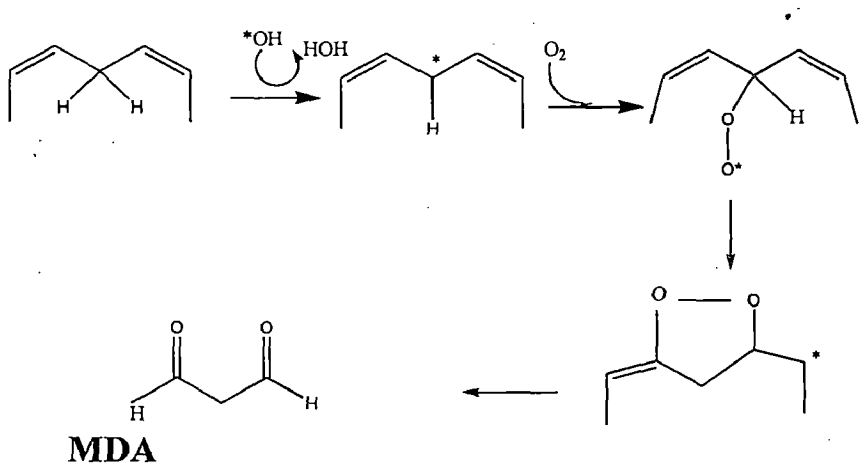


Fig. 1.3 The schematic representation of MDA formation

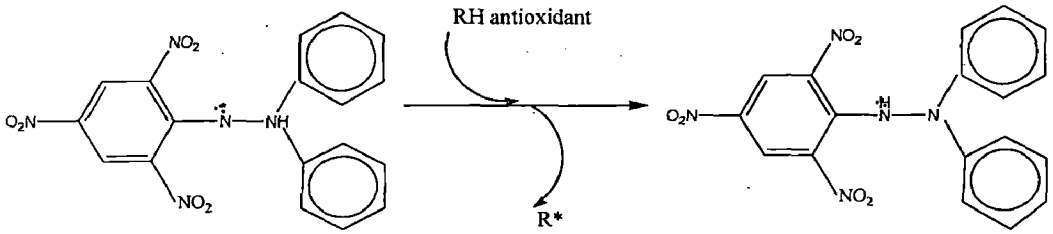


Fig: 2.3 mechanism of inhibition of DPPH• radical by antioxidant

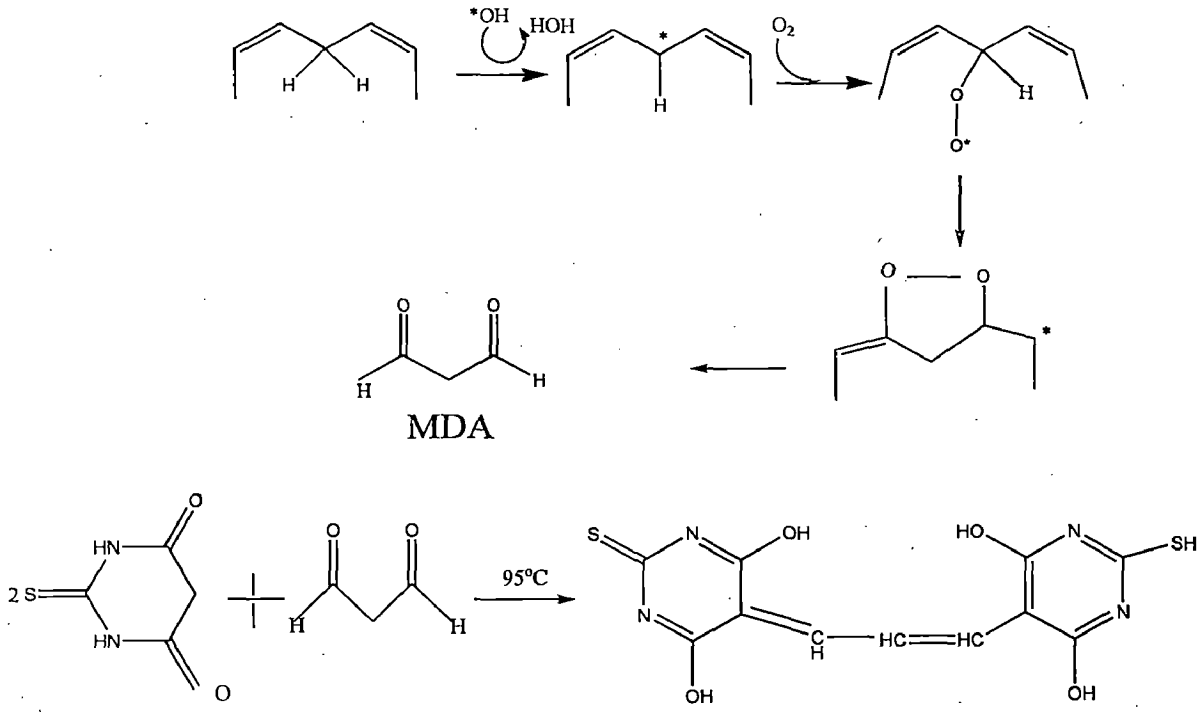


Fig:2.4 Mechanism of lipid peroxidation

Fig:2.1 Soxhlet Extraction

Fig 2.2 Flame photometry

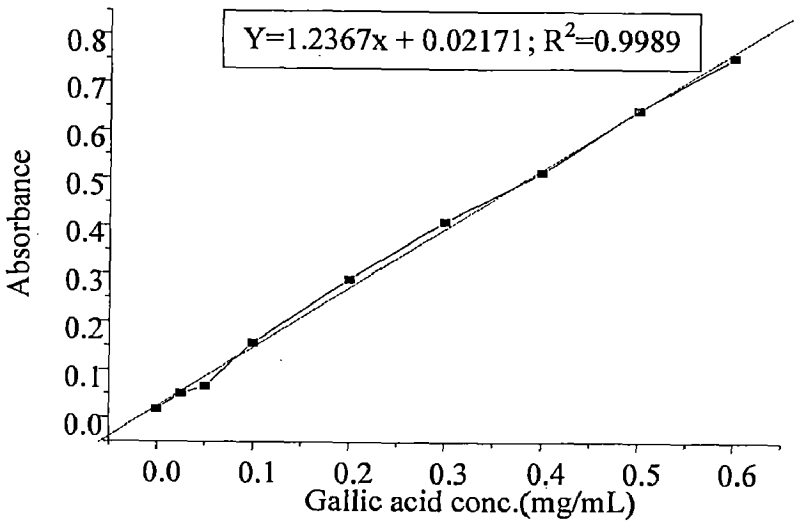


Fig. 3.1 Calibration curve for estimation of Total Phenolic Content

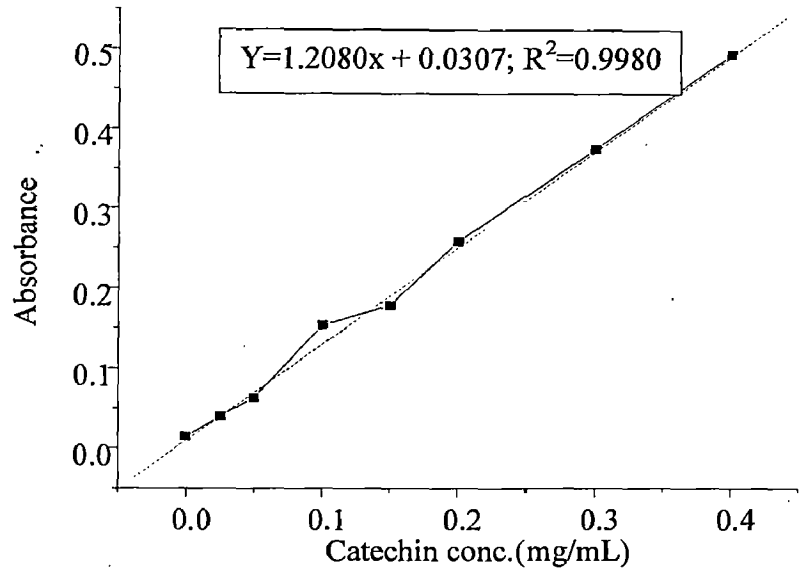


Fig 3.2 Calibration curve for estimation of Total Flavonoid Content

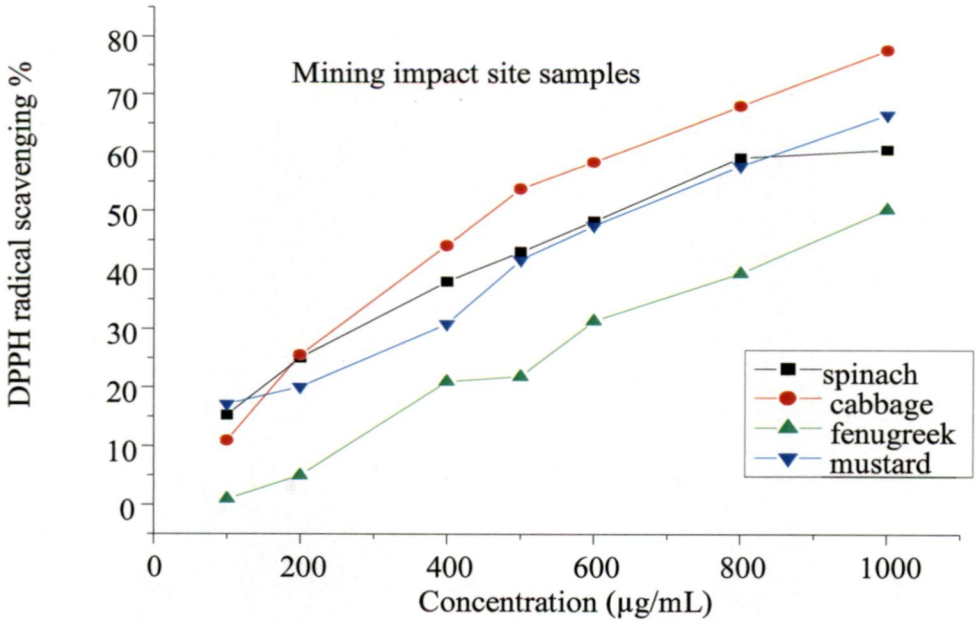


Fig 3.3 DPPH radical scavenging %, representing the trends of DPPH radical scavenging activities by methanolic vegetable plant extract from MIS. Each value is presented as the mean ±S.D. of three replicate determinations. Spinach: S. oleracea; Cabbage: B. oleracea; Fenugreek: T. foenum-graecum; Mustard: B.juncea

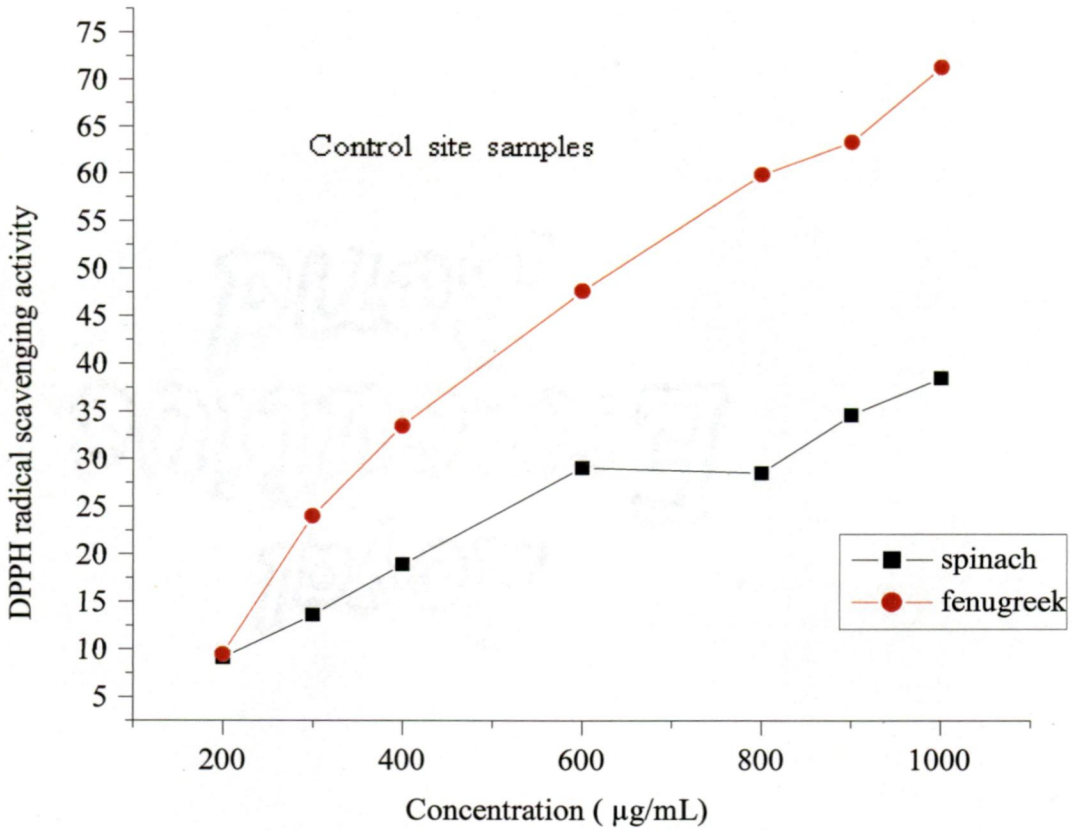


Fig 3.4 DPPH radical scavenging %, representing the trends of DPPH radical scavenging activities by methanolic vegetable plant extract from CS. Each value is presented as the mean ±S.D. of three replicate determinations. Spinach: S. oleracea; Fenugreek: T. foenum-graecum.

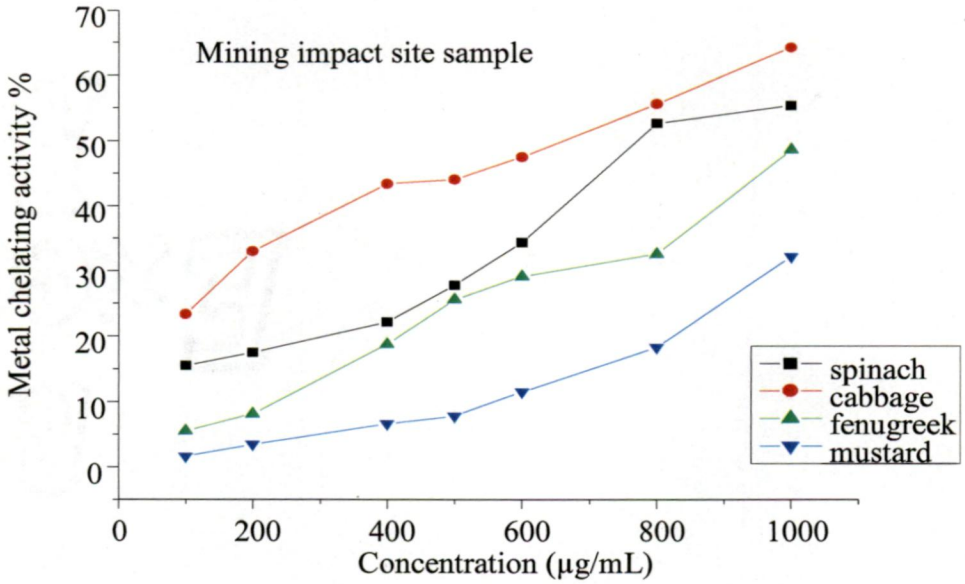


Fig 3.5 Metal chelating activity % of MIS samples representing the trends of Metal Chelating activities by methanolic vegetable plant extract from MIS. Each value is presented as the mean ±S.D. of three replicate determinations. Spinach: S. oleracea; Cabbage: B. oleracea; Fenugreek: T. foenum-graecum; Mustard: B.juncea

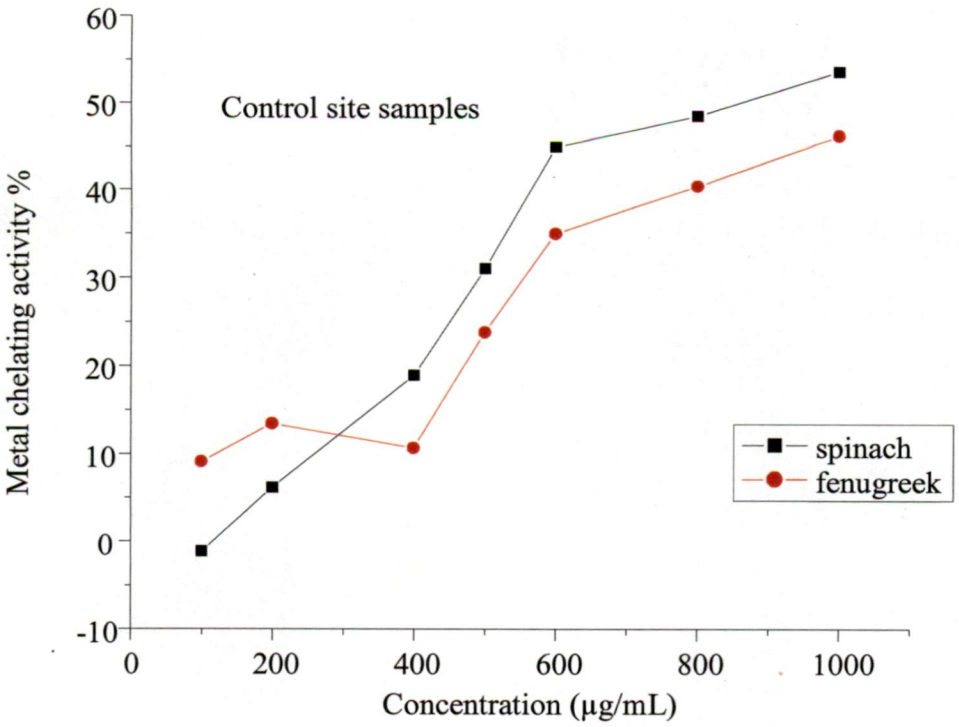


Fig 3.6 Metal chelating activity % of CS samples representing the trends of Metal Chelating activities by methanolic vegetable plant extract from MIS. Each value is presented as the mean ±S.D. of three replicate determinations. Spinach: S. oleracea; Fenugreek: T. foenum-graecum.

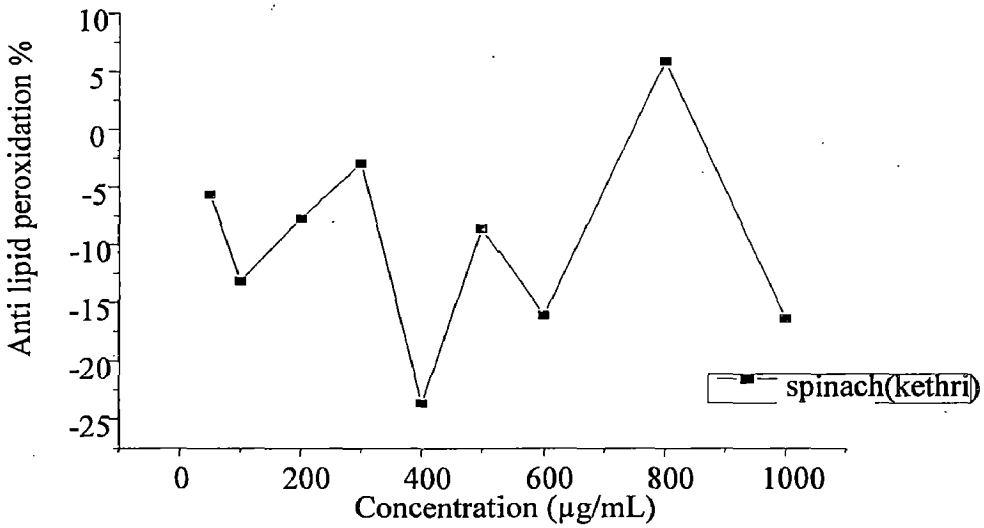


Fig 3.7 Anti-Lipid peroxidation of spinach: S. oleracea.

Annexure -II

TABLES

Table 1.1. List of essential elements, their metabolic functions and deficiency symptoms.[95]Prasad.

Elements	Function	Deficiency Symptoms
Fe	Oxygen and electron transport, constitutes haemoglobin	Anaemia, Stomatitis, Dysphagia, brittle nails
Cu	Constituent of oxidative enzymes, interaction with iron, cross linking of elastin	Anaemia, change of ossification, elevated serum cholesterol, diarrhoea, nervous system damage
Zn	Constituents of enzymes involved in energy metabolism and transcription	Growth depression, poor healing of wounds, sexual immaturity, skin lesions, menstrual problems, change of taste, acuity
Co	Constituent of vitamin B_{12}	Loss of appetite, vitamin B_{12} deficiency
Mn	Constituent of superoxide dismutase, mucopolysaccharide metabolism	Disturbance in bones and cartilage formation, deafness, dizziness
Cr	Potentiation of insulin	Relative insulin resistance, impaired glucose tolerance, elevated serum lipids, fatigue and lack of energy, indigestion
Ni	Interaction with iron absorption, activates enzymes	Anaemia, inconsistent growth
Se	Constituent of glutathione peroxidase, interaction with heavy metals	Endemic cardiomyopathy (keshan disease), premature aging, eye and nerve disorders, infertility
V	Gene regulation and in various enzymatic systems	Several physiological malfunctioning including thyroid, glucose and lipid metabolism
Ca	Essential for formation of healthy bones and teeth, regulates blood clotting , muscle function, nerve transmission	Muscle cramps, brittle bone disease, dental problems
Mg	Help in absorption of other minerals, simulates bone growth and promotes the body use of vitamin B, C, and E	Lack of energy, muscle spams, weakness asthma, cardiovascular disorders
K	Essential for growth, simulates nerve impluses, promotes healthy skin, boosts kidney function, combines with Na to regulate heart beats	Irregular heart beats, dry skin, nervous disorders
As	DNA repair mechanism	Growth depression

Table 1.2: Chemical species and their metal toxicity

Metals	Toxic species	Effects on human system
Cr	Cr^{+6}	Carcinogenicity, teratogenicity, chromosomal aberrations, liver and kidney damage
Ni	Ni^{+2}	Brain, liver, kidney effects, carcinogenic, reproductive effects
Cu	Cu^{+2}	Liver disease, brain damage, bone disease
Zn	Zn^{+2}	Growth retardation, anaemia, reproductive effects
Cd	Cd^{+2}	Kidney dysfunction, osteoporosis, hypertension, reproductive effects
Hg	Methyl-Hg	Neurological, reproductive effects, kidney (as Hg^{+2})
Pb	Pb^{+2}	Neurological haematological, immune, kidney effects

Table 1.3 Experimental parameters for AAS determination

Element	Wavelength (nm)	Concn.range (µg/mL)	Slit width (nm)	Sensitivity (ng/mL)
Cd	228.8	0.2-1.8	0.9	9
Ni	232.0	1.8-8.0	0.4	40
Pb	217	2.5-20	0.9	60

Table 1.4 ICP-MS, Perkin Elmer Sciex (Elan DRC-e) instrumental paratmeters and operating conditions

Instrument parameter	Condition
RF power	1100 W
Carrier gas(inner)	1.00 L/min
Auxiliary gas(intermediate)	1.2 L/min
Plasma gas flow(out)	15 L/min
Nebulizer gas flow	0.9 L/min
Peristaltic pump(sample uptake)	0.9 L/min
Analytical masses	^{55}Mn, ^{56}Fe, ^{60}Ni, ^{63}Cu, ^{66}Zn, ^{111}Cd, ^{208}Pb
Vacuum pressure	$2.5x\ 10^{-6}$ torr
Data acquisition	Peak hopping, dwell time 50 ms, Readings per replicate=1, replicates=3

Table 3.1 Certified concentration values (in μg/g) of Apple Leaves reference material (NIST, SRM-1515)

Elements	Certified values
Fe	83±5
Cu	5.64±0.24
Cr	(0.30)[a]
Cd	0.013±0.002
Ni	0.91±0.12
Mn	54±3
Zn	12.5±0.3
Pb	0.470±0.02

[a] Non-certified concentration

Table 3.2 Elements value determined in Apple Leaves NSIT (SRM-1515) by ICP-MS and AAS

Elements	ICP-MS	AAS
Fe	141.35 ± 37.8	5.455 ± 0.3
Cu	3.25 ± 0.8	94.86 ± 0.04
Cr	8.07 ± 2.0	NA
Cd	0.079 ± 0.04	NA
Ni	4.6 ± 1.0	NA
Mn	29.17 ± 7.2	NA
Zn	57.35 ± 25.6	24.763 ± 0.11
Pb	12.78 ± 15.0	NA

NA = not analysed

Table 3.3 Elemental concentration (μg/g) in dried powdered vegetable samples by ICP-MS

From copper mining site (n=3)

Elements	*S. oleracea*	*B. oleracea*	*T. foenum-graecum*	*B.juncea*
Fe	264.69 ± 25.5	77.5 ± 7.3	254.6 ± 3.13	163.8 ± 41.2
Cu	17.53 ± 2.0	6.84 ± 0.6	18.3 ± 1.4	12.2 ± 2.2
Cr	0.41 ± 0.01	0.37 ± 0.01	0.24 ± 0.17	0.23 ± 0.2
Cd	0.85 ± 1.03	0.04 ± 0.01	0.027 ± 0.01	15.0 ± 21.2
Ni	1.3 ± 0.13	1.4 ± 0.00	0.74 ± 0.5	0.63 ± 0.43
Mn	48.49 ± 3.6	42.6 ± 8.5	41.3 ± 5.1	40.6 ± 5.0
Zn	27.604 ± 0.4	21.0 ± 3.0	26.06 ± 1.1	24.22 ± 1.4
Pb	0.062 ± 0.012	0.18 ± 0.19	0.051 ± 0.02	0.058 ± 0.04

Table 3.4 Elemental concentration (μg/g) in dried powdered vegetable samples by ICP-MS

From control site (haridwar) (n=3)

Elements	*S. oleracea*	*T. foenum-graecum*
Fe	75.0 ± 13.3	104.44 ± 2.9
Cu	19.0 ± 7.9	11.3 ± 1.02
Cr	0.17 ± 0.1	0.3 ± 0.02
Cd	0.022 ± 0.01	0.1 ± 0.11
Ni	0.59 ± 0.27	0.9 ± 0.18
Mn	68.7 ± 9.7	41.7 ± 1.0
Zn	32.2 ± 1.5	25.3 ± 0.71
Pb	0.023 ± 0.01	0.044 ± 0.02

Table 3.5 Elemental concentration (μg/g) in dried powdered vegetable samples by AAS

From copper mining site (n=3)

Elements	*S. oleracea*	*B. oleracea*	*T. foenum-graecum*	*B.juncea*
Fe	237.81 ± 0.62	58.47 ± 0.13	290.3 ± 0.09	224.2 ± 0.27
Cu	16.1 ± 1.2	5.33 ± 0.2	25.4 ± 0.07	15.31 ± 0.34
Zn	55.75 ± 0.2	32.06 ± 0.44	59.8 ± 0.00	66.9 ± 0.18

Table 3.6 Elemental concentration (μg/g) in dried powdered vegetable samples by AAS

From control site (haridwar) (n=3)

Elements	*S. oleracea*	*T. foenum-graecum*
Fe	113.2 ± 0.42	139 ± 0.18
Cu	10.5 ± 0.008	6.2 ± 0.018
Zn	89.4 ± 0.62	48.4 ± 0.4

Table 3.7 Alkali elemental concentration (μg/g) in dried powdered vegetable samples by Flame photometry method

From copper mining site (n=3)

Elements	*S. oleracea*	*B. oleracea*	*T. foenum-graecum*	*B.juncea*
Na	3390 ± 35.7	1755 ± 18..4	1551.25 ± 13.8	9388.12 ± 87.3
K	2317.5 ± 21.5	1945 ± 20.8	1661.25 ± 20.6	1725 ± 20.4

Table 3.8 Alkali elemental concentration (μg/g) in dried powdered vegetable samples by Flame photometry method

From control site (n=3)

Elements	*S. oleracea*	*T. foenum-graecum*
Na	1327.51 ± 15.6	2295 ± 25.4
K	2061.25 ± 22.3	1510 ± 13.2

Table 3.9 Total heavy metal concentration (in mg Kg^{-1}) of dried soil sample from Mining impact site (MIS) and Control site (CS) (n=3)[131]

Elements	Mining impact site	Control site
Fe (%)	3.40 ± 0.83	2.32 ± 0.94
Cu	763 ± 19.3	26.4 ± 2.9
Cr	102.8 ± 2.9	33.4 ± 1.4
Cd	3.6 ± 0.4	3.5 ± 0.2
Ni	16.2 ± 1.5	11.6 ± 1.1
Mn	268.8 ± 5.7	276.5 ± 7.9
Zn	111.2 ± 9.3	74.4 ± 4.3
Pb	20.6 ± 1.9	12.5 ± 1.3

Table 3.10 Bioaccumulation factors (BAFs)of heavy metals in vegetables plants collected from MIS (mining impact site) and CS (control site) (by ICP-MS)

Element	*S. oleracea (MIS)*	*S. oleracea (CS)*	*T. foenum-graecum (MIS)*	*T. foenum-graecum (CS)*	*B. oleracea (MIS)*	*B. oleracea (CS)*	*B.juncea (MIS)*	*B.juncea (CS)*
Fe	0.78	0.32	0.75	0.45	0.23	NA	0.48	NA
Cu	0.02	0.72	0.023	0.43	0.008	NA	0.02	NA
Cr	0.004	0.005	0.002	0.01	0.004	NA	0.002	NA
Cd	0.24	0.006	0.01	0.03	0.01	NA	4.2	NA
Ni	0.08	0.05	0.05	0.07	0.09	NA	0.04	NA
Mn	0.18	0.25	0.15	0.15	0.16	NA	0.15	NA
Zn	0.25	0.43	0.23	0.34	0.19	NA	0.22	NA
Pb	0.002	0.002	0.002	0.003	0.01	NA	0.002	NA

MIS= Mning Impact Site CS= Control Site

Table 3.11 Total Phenolic and Total Flavonoid Contents values in MeOH extract of different of vegetable plants from MIS and CS.

Plant species	*Total phenolic content (mg GAEs/g extract)*		*Total flavonoid content (mg CEs/g extract)*	
	MIS	*CS*	*MIS*	*CS*
S. oleracea	18.29±3.7	18.05±1.3	20.18±1.4	16.79±0.02
B. oleracea	22.63±0.02	NA	9.75±2.2	NA
T. foenum-graecum	15.77±2.2	19.14±0.5	14.48±0.91	15.46±0.2
B.juncea	12.82±1.8	NA	13.84±0.68	NA

Table 3.12 IC_{50} (µg/mL) values of DPPH• free radical scavenging activity, metal chelating activity and inhibition of lipid peroxidation in MeOH extract of different vegetable plants from MIS and CS.

Plants species	IC50(µg/mL) DPPH scavenging activity		IC50(µg/mL) metal chelating activity		IC50(µg/mL) lipid peroxidation	
	MIS	CS	MIS	CS	MIS	CS
S. oleracea	637.4	>1000	767.48	830	NA	NA
B. oleracea	470	NA	670	NA	NA	NA
T. foenum-graecum	1000	650	>1000	>1000	NA	NA
B.juncea	660	NA	>1000	NA	NA	NA