Review Paper:

Heavy metal-induced genotoxicity in Flax (*Linum usitatissimum* L.) and its effect on Environment and human health

Dhall Subhadarshani, Biswal Bhagyashree, Jena Biswajit, Giri Alok Kumar and Acharya Laxmikanta*
Molecular Biology and Genetic Engineering Lab, Centre for Biotechnology, School of Pharmaceutical Sciences,
Siksha 'O' Anusandhan (Deemed to be University), Bhubaneswar, Odisha, INDIA
*laxmikantaacharya@soa.ac.in

Abstract

Environmental pollution is on the rise all around the biosphere. Heavy metals such as Pb, Cr, Mn, Fe, Ni, Cu, Zn, Cd and Hg contaminate soil, posing substantial environmental impacts because these metals are non-essential and poisonous to plants and animals as well as having a significant detrimental effect on human health. Heavy metals are released and enter the plant system through a variety of physiological processes, affecting the growth and development of the plant. Heavy metal concentrations in the environment are variable as a result of numerous activities and they become harmful when they exceed allowed levels. The harmful effect of heavy metals on plants increased as the concentration of heavy metals in the soil increased.

Heavy metal contamination has been reported in oilseed crops; however, no such comprehensive review is available in Flax. In the present review, the effect of heavy metal on flax has been thoroughly analyzed. Flax (Linum usitatissimum L.) is a self-fertilizing annual plant that is used for its fiber or seed oil. It is rich in alpha-linolenic acid, an omega-3 fatty acid, as well as the lignan-Secoisolariciresinol diglucoside and fiber. Through their anti-inflammatory, anti-oxidative and lipid regulating capabilities, these chemicals provide bioactivity that is beneficial to the animal and human health. This review describes the accumulation of heavy metals on the growth and development of flax, the advantages of ingesting flax seed or its bioactive components and the beneficial effect of flax on human health.

Keywords: Heavy metals, Flax fiber, Oilseed Crop, Alpha-linolenic acid, Nickel, Cobalt.

Introduction

Flax (*Linum usitatissimum* L.) is one of the most widely adopted multiuse crops. It is a member of the Linaceae family which includes 22 genera and two subfamilies: the Linoideae and Hugonioideae. Among the Linoideae, *Linum* is the largest genus with more than 300 different species[2] and is cultivated for both food and fiber. It is also called Linseed and belongs to the genus *Linum*, the family is Linaceae, a division is Magnoliophyta and Order is Malpighialesa. Carl Linnaeus gave the Latin or scientific name *Linum usitatssium* in his book "Species Plantarum" (Linnaeus; 1857)[25]. Flax is also known as *Linum sativum* Hasselq. It is cultivated throughout North America and Asia. Flax *(Linum usitatissimum)* was originally cultivated in Mesopotamia. Flax was the first fiber crop grown 10,000 years ago in Egypt and Samaria[15]. It is native to central Asia and the Mediterranean region. Flax is an herbaceous annual crop.

The annual production of Flax was 3.06 million tons all over the world and Canada is the largest producer of flax contributing around 38% of total production. Flax mostly grown in Maharashtra, Chhattisgarh, Madhya Pradesh and Bihar in India[17]. The Blue flax (Linum perenne L.) is a perennial herb that is nonclonal[35]. Flax is a self-fertilizing plant that is commonly grown in temperate regions. Fiber flax cultivars, which are taller, less branching and grown at low temperatures, are one form of flax produced.

North Asia and Eastern Europe are the primary regions where it is grown. Fiber flax is being harvested on around 500,000 hectares across the world. After cotton and jute, flax fiber is the third most commonly used plant fiber. Flaxseed, also known as linseed, is a kind of seed that is shorter in length, has bigger seeds and is more branching and is cultivated in warmer climates such as India, China, Canada, Argentina and the United States. Flaxseed is currently grown on 3 million hectares across the world and the production of vegetable oil is around 1% of the total world[14]. Flax has been utilized commercially to make a variety of value-added goods, either directly or after processing.

Flaxseed has been consumed in various forms as a dietary ingredient and for its therapeutic benefits since it was first cultivated about 5000 BC. The demand for flax has expanded dramatically as people have become more aware of the link between health and food[27].

Many illnesses such as hyperlipidemia, colon tumors, breast cancer and atherosclerosis, are helped by linseed oil[56]. Fiber, seed oils, feed and attractive plants were all derived from Linum species. Flaxseed oils contain omega-3 fatty acids and anti-cancer compounds, whereas lignans have been discovered in the blossoming aerial parts of these species[47]. Even though linseed (Linum usitatissimum) is high in linolenic acid, one of the richest dietary sources of α-linolenic acid and is also a strong source of soluble fiber gum or mucilage, it is still underutilized in the human diet[6].

Growth and Morphology of flax: Flax plants are dicot plants. The flax plant's life cycle includes a 45-60 days growth stage, a 15-25 days blooming period and a 30-40 days maturity period. The leaves are simple, greenish, linear-lanceolate and glaucous having a width of 3mm and a length of 20-40mm. The average height of the plant varies from 0.9 to 1.2 meters with slender stalks of 2.5 to 4 mm in diameter with the majority of the branches located near the top. The roots are shallow and fibrous and the major roots are split into several lateral roots with numerous branches. The flowers are hermaphrodite and hypogynous and are borne on stems and grow from the branch tips.

They have five petals, are normally blue but can be pinkish or white depending on the species and are hermaphrodite and hypogynous. Seeds are like dry capsules and glossy brown. These plants are grown for the collection of seeds for oil and fiber. The plant is adapted to cool moist climates and grows fairly in well-drained soil.

After two weeks, as the seed capsules develop, the plants become yellow, the fiber dissolves and the plants turn brown. Flax plants grow well in calcareous soil because they may modify the rhizosphere environment, increasing the solubility of soil nutrients and lowering nitrogen and phosphorus availability, as well as zinc and iron availability[37].

Factors affecting plant growth

The main factors affecting the growth of plants include:

- **Soil:** The soil is a combination of organic matter formed from the decomposition of plants and animals as well as minerals derived from the rock beneath the soil or delivered by wind or water. It is an essential substrate for plants growth[32]. Soil supplies nutrients, supports a network of air and water to the roots of the plant. Mostly well-drained, non-sticky, sandy and loamy soil types are preferred for the cultivation of flax.

- **Light:** Light can affect plant morphology. Depending on whether they are shade leaves or sun leaves, leaves on the same plant can differ from one another. Depending on the light intensity, duration and wavelength, the plant's response to light will vary[53]. Plants respond to light of wavelengths ranging from 300-800 nm. Flax plants were well grown in a sunny and sheltered position and they did not admire excessively hot weather.

- **Water:** Plants require water to survive. Water makes up around 70% of the human body, while plants are more like 90% water. Without water, plants are stressed and die. Water acts as a transmission medium within the plant and also functions as the cell's solvent system.

  Water is one of the raw materials needed for photosynthesis to make new compounds. Establishing flax flowers is appreciated on a moist soil. It will not need too much watering. A thin layer of mulch helps to retain moisture and it can also help weed control[50].

- **Temperature and humidity:** The plants need the temperature to trigger or delay the life processes. The warmth stimulates the process of growth and germination. Warmer temperatures accelerate the chemical reactions in the plant's cells that trigger photosynthesis, transpiration and respiration. Plants grow more quickly during hot weather, slow down, or even go dormant during cooler weather. In the atmosphere, water is present in the form of invisible water vapor, known as humidity. Generally, 40-60% of humidity is suitable for most crop plants. If humidity is high in the atmosphere, there is a chance for the outbreak of pests and disease. Flax plants and flowers are not grown in overly hot and dry regions. They have thrived in damp and cool climates[9].

Bioactive constituents in flaxseed

Due to the inclusion of different bioactive components, flax, flaxseed and oil are considered beneficial[13]. Flaxseed contains 20-40% oil, 5-6% palmitic acid, 3-6% stearic acid, 14-18% linoleic acid, 19-29% oleic acid and 45-52% alpha-linolenic acid (ALA)[49,56]. Flax seeds are high in monounsaturated fatty acids like oleic acid. Although both ALA and linoleic acid are necessary fatty acids, both can be produced by the human body and must be received from the diet[36]. Flaxseed protein and peptides produced from flaxseed exhibit physiological properties such as antibacterial activity, suppression of angiotensin-converting enzyme, anti-diabetic and antioxidant capacity[59]. Previous research has revealed that 2-5 tablespoons of milled flax, which contains 4-10 grams of alpha-linolenic acid (ALA), or 1-3 tablespoons of flax oil, which provide 3-20 grams of ALA, should be included in your normal diet. Flaxseed is a good source of oil with polyunsaturated fatty acids, digestible proteins and high dietary fiber content. Because of the possible health benefits linked with its bioactive ingredients, it is the curiosity of nutritional and medical researchers[17].

Flaxseed is rich in omega-3fatty acid and lignans, a class of phytoestrogens. It has nitrogenous secondary metabolites called cyanogenic glycosides (CG) and it also contains cyclic hydrophobic peptides of amino acid residue known as cyclolinopeptides (CL) [39]. Flaxseed protein contains higher amounts of glutamic acid, aspartic acid, arginine and amino acids. The most biologically active and clinical studies of flax have focused on extracts containing alpha-linolenic acid and lignin[48]. The bioactive constituents of flax seed and their respective biological activity were depicted in the table 1.

Flax fibers have thermoplastic properties. Polyhydroxybutyrate (PHB) is a fatty acid synthesized by stem cells. PHB is a thermoplastic agent that is non-toxic, water-soluble and biodegradable and has chemical and physical properties that are identical to the petroleum-derived polymer polypropylene[14].

Table 1
Components and function of Flaxseed

COMPONENTS	FUNCTION
ALA (Alpha linolenic acid)	Reduce cardiovascular risk (CVD), low-density lipoprotein, improve insulin sensitivity and reduce HDL cholesterol levels of menopausal women.
Omega-3-fatty acid	Improves heart function, reducing cholesterol, reduces CVD risk markers.
Phenolic complex	Reduced the plasma glucose
Secoisolariciresinol diglucoside (SDG)	Decrease oxidative stress
Omega-6-fatty acids	Reduced chances of colon cancer initiation
Docosahexaenoic acid (DHA)	Increase the memory, Reduce depression symptoms.
Oligosaccharides	Regulation of gastrointestinal function, liver protection, antitumor

The flax plant has nitrogenous components including protein, amino acids, enzymes, polyamines and cyanogenic glycosides. The differentiation in nitrogen compounds occurs during the germination of flaxseed[57]. The flax protein is in the form of an amino acid pattern, which is similar to that of most nutrient soybean protein.

Uses of Flax

Industrial use: Flax has also been used for commercial purposes (Figure 1). The oil extracted from the flaxseeds is employed for the production of paints, printing inks, vegetable oil and linoleum[56]. Sometimes taken as nutritional supplements and its by-products are used in animal feeds. Flax has been considered a very useful plant and also for the production of oil, fiber and traditional medicine[51]. Flaxseed oil has a high content of ALA which readily oxidizes and polymerizes. Because of this characteristic, it can be used for industrial purposes but it cannot be used as a direct replacement for canola or corn oil. Flax oil has such unique characteristics that continuous efforts are being made to elucidate its fatty acid profile[25].

Nowadays the use of flaxseed in human diets has increased throughout the world. Flaxseed is mostly used to create oil for industrial applications such as paints, linoleum, varnishes, inks and herbal cosmetics, but it is also employed in the pharmaceutical industry. Flax fibers are used in the textile industry. It plays a vital role in functional foods for nutrients like protein, soluble fiber, minerals and vitamins[61]. Flax comes in two varieties: brown and yellow. They have similar nutritional qualities and have the same amount of omega-3 fatty acids. Solin is yellow or pale flax (*Linum bienne*) that has a distinct oil profile and is low in omega-3 fatty acids. Paints, varnishes, fiber and animal feed all employ brown flaxseed[13].

Flaxseed is an oilseed and besides nutrition, there are three main reasons for this: the first is that it is high in n-3 polyunsaturated fatty acids (Omega 3 fatty acid) the second is that it is high in dietary soluble and insoluble fiber and the third is that it contains phytoestrogens and high lignans, which act as anti-oxidants. Flaxseed also acts as an anti-arrhythmic, anti-atherogenic, anti-thrombotic and anti-inflammatory agent in addition to improving vascular function[16]. Flax fibers are stronger enough for the manufacture of shoe threads, sewing threads and button threads. It is also utilized as a raw ingredient in the paper industry for printing banknotes and cigarette paper.

Flax fibers based on the polyhydroxybutyrate polymer could be a good option for the environment and biodegradable renewable raw material as well as a plastics replacement. Flax fibers are inexpensive and have good chemical properties. Due to its unusual characteristics and tensile strength, it was the first fibrous crop to be spun and woven into textiles. Many pigments like cellulose, wax, lignin, hemicellulose and pectin, are found in its fiber[45]. Kerchiefs, bedding, curtains, drapes, pillow covers, wall coverings, various ornamental materials, coats and traditional outfits are all made of linen. Flaxseed oil can be used as a supplement to other cadmium poisoning treatments, as well as utilized directly or as a dietary additive[26]. Flax can be used for phytoremediation and is a non-toxic raw material.

The beneficial effect of Flax on Human Health: Flaxseed has high fiber content with high energy density. It is quickly becoming one of the most important phytochemical sources in the functional food industry. Flaxseed contains about 40% oil, 30% dietary fibre, 20% protein, 6% moisture and 2% ash[5]. Flaxseed is high in soluble and insoluble fibers, which help the digestive system work efficiently. Mucilage, which is the most soluble fiber in flax, is a powerful cholesterol-lowering agent. Insoluble fiber aids in the prevention of constipation and the control of bowel motions. Flax fiber is a silky, glossy and flexible fabric that is much stronger than cotton but less elastic. Lignan is an antioxidant in humans and the flaxseed plant has 800 times more lignans than other plant seeds, making it the richest source of plant lignans. Because lignan is transformed into phytoestrogens chemicals, which impede the activity of hormone-sensitive malignancies, consuming 2-4 tablespoons of flaxseed can help prevent the growth of malignant tumors[25]. Flaxseed contains detectable levels of cadmium, it activates the estrogen receptor. Omega 3 fatty acids reduce female offspring's later mammary tumorigenesis [7].

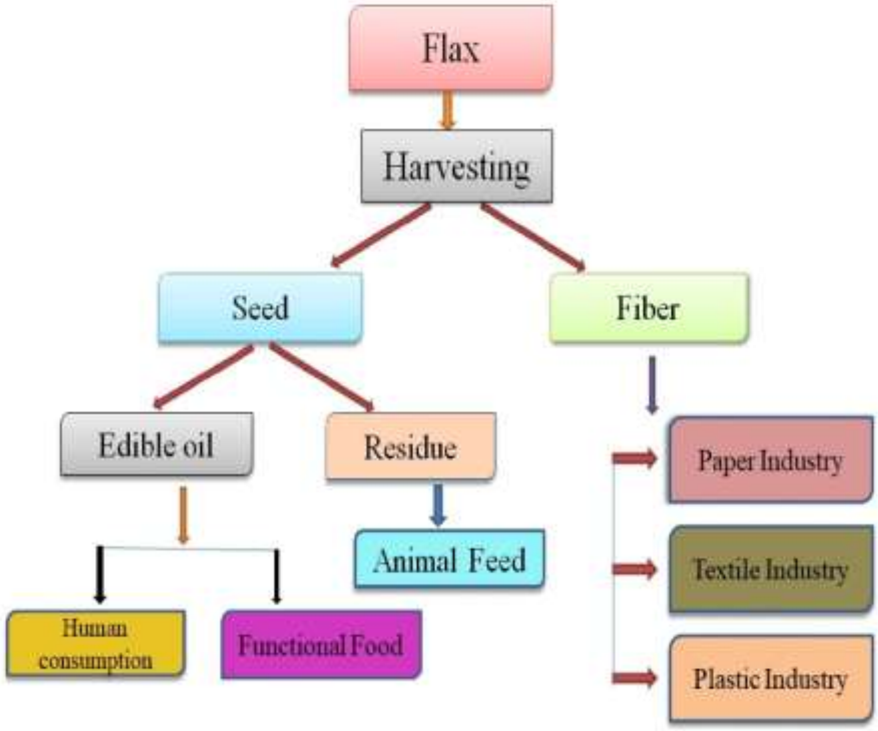

Fig. 1: Schematic diagram representing the usage of flax

Flaxseed contains peptides such as cyclolinopeptide with bioactivities which decrease the risk factors of cardiovascular disease (CVD), inhibit the human malarial parasite and have strong immunosuppressive and antimalarial activities, well-controlled type-2 diabetes, chronic diseases, certain cancer, reduce cholesterol, protection against ischemic heart disease by improving vascular relaxation, protection of kidney and metabolic control in diabetes mellitus[13]. Flaxseed has been shown to lower the risk of breast cancer, but flaxseed has also been shown in several trials to lessen the incidence of prostate cancer by reducing inflammation. The omega-3 fatty acids aid in the enhancement of physiological and molecular properties in retinal tissue[8]. It acts as an anti-inflammatory in chronic metabolic disease[63]. The consumption of flax is very effective in the menstrual cycle of women[41].

Flaxseed protein may also help in maintaining the glucose levels in the blood because of its interaction with the gums and also by stimulating insulin secretion resulting in reduced glycaemic response[38]. Colds coughs and urinary tract problems have all been treated with linseed tea. Linseed oil may also be used as a laxative and it can be used to soothe burns when mixed with lime water. It is well-known as a honey-based natural cosmetic for eliminating face blemishes. The flax plant is extremely adaptable in every way.

The toxic effect on humans: The major nutritional components of flax, which have been shown to interfere with tumor start and progression in animals, have a detrimental effect. Excess consumption of flaxseeds without enough liquids can lead to intestinal blockage, so people suffering from intestinal conditions should avoid consuming flaxseed. It is particularly dangerous for Scleroderma patients.

Accumulation of heavy metal on Flax: Heavy metals accumulate in plant tissue as plants absorb increasing amounts of heavy metals from soil that has been contaminated with heavy metals. Heavy metals enter the human body via the food chain and humans are only exposed to heavy metals through the ingestion of contaminated plants. When heavy metals are not absorbed by the body and accumulate in the soft tissues, they become dangerous. In comparison to locations with lower heavy metal concentrations, flax plants growing organically in metal-rich soils resulted in significantly higher accumulation.

Heavy metal from the soil is absorbed and accumulated most effectively by this crop. The distribution of heavy metal along plant bodies seems to be effective in decreasing the following order: root > stem > leaves > seed[3,19]. Linseed plants can accumulate high levels of Cd and Zn in natural fields. The mechanism of heavy metal uptake by plant from soil was shown in figure 2.

Sources of Heavy Metal presence in the Environment: Heavy metals are classified as essential or non-essential in the environment. Plants require heavy metals such as cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni) and zinc (Zn) to grow and develop. Heavy metal accumulation is harmful to ecological, nutritional and environmental balance. Biogeochemical processes have been disrupted as a result. The term "Heavy metal" is defined as the chemical or metallic element which has a relatively high density and is toxic or poisonous even at a low concentration. The density of heavy metal is more than $5g/cm^3$ [28].

Heavy metal reduces crop productivity capacity by inducing various effects in physiological processes in the plants like seed germination, accumulation and remobilization of seed during the germination process, development and growth of the plant and photosynthesis. Plants utilize these micronutrients to complete their life cycle. Increased heavy metal concentrations in plant tissue have both direct and indirect toxic effects. The direct harmful consequences of oxidative stress are generated when cytoplasmic enzymes are inhibited, resulting in cell structural destruction. The replacement of critical nutrients at the plant ion exchange site has an indirect harmful impact on the plant. This ion has a strong impact on the pace at which phytotoxicity is revealed, as well as the rate at which different enzymes and proteins are arrested. The indirect influence might outweigh the direct effects. The plant can survive biotic and abiotic stress.

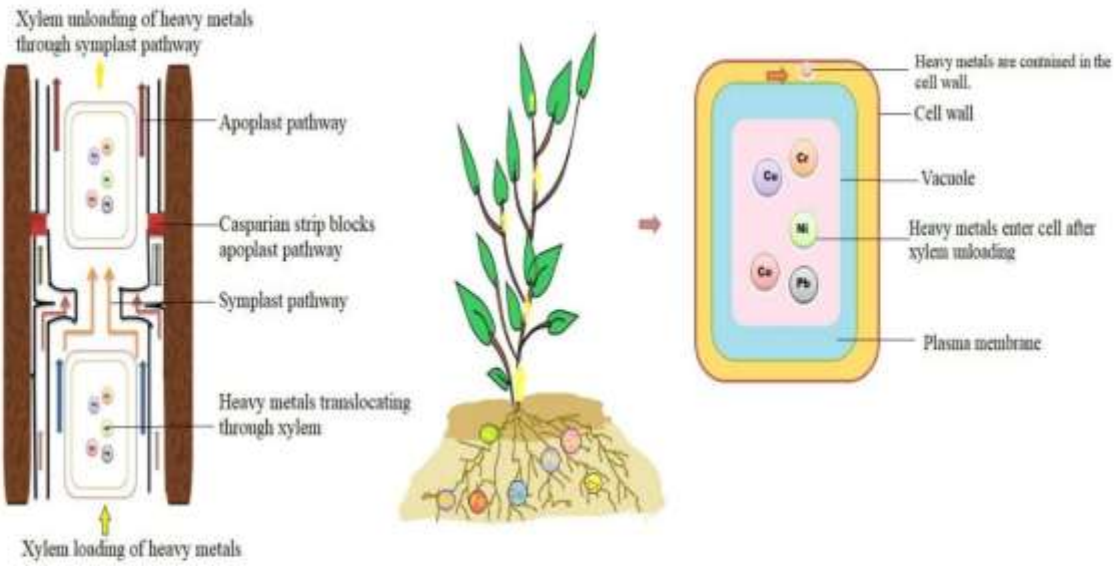

Fig. 2: Plant Heavy Metal Uptake Mechanism

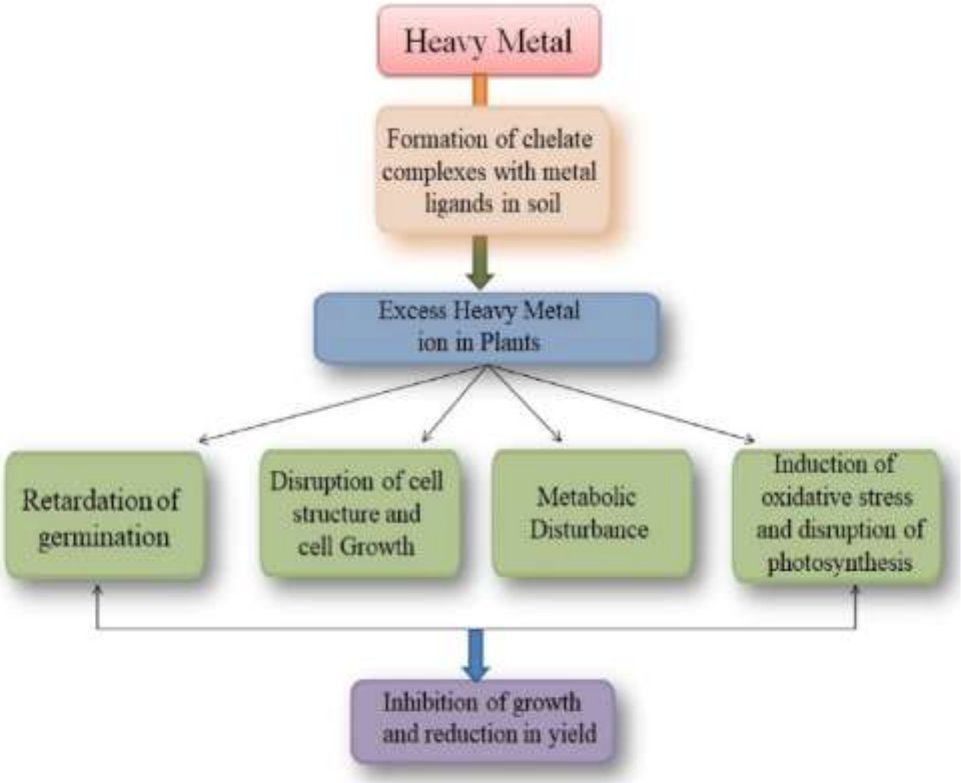

Fig. 3: Diagram depicting the mechanism of heavy metal accumulation and its hazardous effect on plants.

Effect of heavy metal stress on plants: Heavy metal stress is one of the major stresses having a significant negative impact on crop yield and growth. Chemical element accumulation on stressed plants is influenced not only by their absolute content in the soil, or by soil fertility, pH and organic matter content. The heavy metal accumulation is dependent between metal content in the soil and the crops [23]. Heavy metals are steadily released into the terrestrial environment by natural phenomena and also an artificial phenomenon. Plants require trace elements for basic metabolic activities, however in greater quantities, these metals are dangerous and poisonous. The excessive metal concentration in contaminated soil can affect decreased soil fertility and soil microbial activity and can decrease yielding capacities[41,63]. Heavy metals are promoting protein denaturation and increase the activities of proteases, RNase and DNase enzymes [62]. The hazardous effects of heavy metal accumulation in plants are depicted in figure 3.

Sources of Nickel presence in Environment: Nickel is a heavy metal that is found in nature. Its usage in several sectors such as industrialization, adoption as chemical fertilizer and sewage sludge, has increased the nickel concentration in the environment, posing a threat to the ecosystem. Nickel is found in soil and water in tiny amounts. It makes up 3% of the earth's makeup. Nickel is found in the form of Nickelous Ni (II) or (Ni^{+2}) in the soil, even though it is present in the form of $[Ni\ (H_2O)_6]^{+2}$ in the soil solution. Nickel exists both in soluble and insoluble compounds in soils, fumes and water. In ecosystem, Ni enters through different sources including nickel-plated objects, soil, industrial wastes, water sources, agricultural activities. A high amount of Ni is present in surface water.

Varying water bodies have different amounts of Ni. Ni concentrations in drinking water are fewer than ten grams per liter. The environment also receives Ni from the air. Ni concentration is modest (00001-0.003g/m^3). The distribution of Ni in the soil is uniform. Ni is uniformly distributed in soil surface but it varies in their different concentration which is finally affecting crop growth yield, quality and quantity of the development. Over the ecosystem, Ni is considered a significant contaminant. It produces emissions from both natural and man-made sources. The concentration of Ni increase in the soil is due to the burning of fossil fuels, industrialization of many types of electroplating, municipal waste, vehicular emission, mining of metal, ore extraction, electronic batteries and applications of many Ni fertilizers and chemical fertilizers as the main sources of increase of Ni toxic in the environment[31].

Effects of Nickel on the plant: Nickel is more hazardous for our environment, so it is critical to comprehend its biological role as well as its harmful effects on plants. Plant development is influenced both positively and negatively by nickel. The number of nutrients in the growing media will determine how long it takes for the plant to reach maturity. On one hand, Ni is required for healthy plant development, enzymatic activity, nitrogen metabolism, iron absorption and several metabolic activities. On the other hand, increased Ni levels limit seed germination, root and shoot growth, biomass accumulation and crop yield or final production. The excess concentration level of Ni leads to toxicity in the plant. It produces chlorosis and necrosis in plants, as well as inhibiting numerous physical processes and oxidative damage. Because of its various biological roles in plants, Ni is considered an important plant micronutrient. Ni shortage affects plant growth and development, causing senescence, as well as N assimilation and iron absorption in plants.

Ni also participates in a variety of metabolic processes including hydrogen metabolism, methanogenesis and acetogenesis. It plays a key function in the production of phytoalexin and plant defense against various stressors. If an excess amount of Ni is associated with plant, adverse effects occur like reduction in germination, plant growth, cell division, biomass production, nutrient absorption and effect in photosynthesis, transpiration, leaf chlorosis and necrosis. Ni stress reduces crop yield and quality of output as a result of these harmful consequences. Ni deficiency in leguminous crops causes leaf damage as well as a reduction in root growth and nodule formation.

In several leguminous crops, a trace amount of nickel is required for nodule growth and hydrogenase enzyme activity. The hydrogenase enzyme's activity is reduced when Ni is deficient. Ni is also important in the activity of several enzymes. It has been proven to be a significant stimulator of plant growth and development at low concentrations.

Plants absorb Ni mostly by active and passive diffusion through the root system. The process of metal absorption by plants is affected by the pH of these soils, the concentration of the soil and the solubility of the metal present in the soil solution. Plants that have a higher Ni content are more disease resistant. Ni is also carried to other sections of the plant such as the fruits and seeds via phloem tissues. It can move easily from the root to the top sections of plants because it easily moves through phloem and xylem vessels. In the case of low soil pH or acidic soil, the symptoms of Ni toxicity can be monitored easily because Ni becomes more soluble and mobile.

Ni is able to form a complex with soil colloid particles; as a result the soil readily converts to a crystal lattice form with different nutrients in the solid phase. Plants readily absorb the available form of Ni from the soil. Naturally serpentine soils contain a high concentration of heavy metals including Ni. Ni also affects plant physiological processes including enzyme activities. The direct and indirect impacts of heavy metals on enzyme activity are difficult to distinguish. If ion-induced imbalances and nutrient transport have unintended repercussions, heavy metals also block enzymes by binding with the SH groups of proteins, resulting in enzyme inactivation[52].

Accumulation of Nickel in plant: Generally in plants, Ni stress is mainly accumulated in the roots, although it may also be found in the top portion of the plant in some species. As a result, plant Ni absorption and accumulation are influenced by the species, the pH of the soil and the amount of Ni in the soil. The plant system is also inserted through the leaves by Ni. When Ni solution was sprayed on leaves of tomato, soybean and oat plants, more than 37% of the applied solution was carried to other parts of the plant such as the root, stem and seeds. Ni uptake in plants occurs by diffusion from the root system to the shoots and then to the leaves via transpirational flow through the xylem parenchyma and arteries in both active and passive conditions.

Furthermore, 80 percent of Ni is absorbed by plants in the root vascular cylinders, while less than 20% is absorbed in the cortex [18]. Plant stems, leaves and leaf organelles all have different Ni distributions. Ni concentrations in the vacuole and cytoplasmic fluid may be high, while amounts in the chloroplast, mitochondria and ribosome are low. In hyperaccumulating plant species living on Ni-polluted soil, Ni is eliminated from embryonic tissue and the reproductive system develops. Ni accumulated in the roots and shoots of finger millet, pearl millet and oats and there was evidence of its influence on plant physiology[16].

Under the treatment of Ni stress on maize plants, leaves condition became chlorotic at low concentration and necrotic at high concentration[5]. Ni is readily absorbed by rice roots, So, in the high concentration of Ni, toxicity was troublesome for rice crops in many areas[34]. The toxic effect of Ni in plants includes a decrease in the morphological nature like shoot length, root length and reduction of leaves. In hyperaccumulation of Ni i.e. $Ni_{0.01}$ and $Ni_{1.00}$ effect on the leaves of soybean and Mungbean is observed.

In the case of barley, Ni stress was affected under 200mM and it did not survive in 400mM Ni concentration. Under 100mM concentration, Ni stress decreased fresh weight of wheat's root. In canola, the plant's roots and shoots elongated as a result of high Ni content. The fresh weight of the sunflower plant's shoot falls when the Ni stress concentration rises from 10 to 40 mg^{-1}. The reduction of these crops was due to the accumulation of Ni in these plants' leaves [20]. In the case of a plant, Ni stress causes metabolic disorders, reduces pectin binding to cell walls and enhances peroxidase activity in cell walls and intracellular areas required for the lignification of polysaccharides containing ferulic acid. It also inhibited cell division, so it decreases cell growth. The excessive accumulation of Ni decreases the level of chlorophyll contents, which ultimately reduces photosynthesis in plants[18].

In the case of tomato Plants, the concentration of 10 mg/l of Ni in the solution may be of limited value in a higher concentration of Ni stress reducing the number of flowers and fruits [6]. With rising Ni levels in the nutrient solution, the effects of Ni stress in barley plants increase the concentration of Cu in shoots and roots. The concentration of Mn in these treated plants' roots and shoots dropped as the Ni content in the nutrient solution increased, but there was no effect on the leaves. The content of Zn in shoots and roots was reduced when the plants were exposed to Ni. As the Ni content in the nutrient solution increased, the Fe concentration in the treatment plant's shoots fell.

Plants cultivated at 100 mM Ni had a deficiency risk below the dangerous limit[42]. In the case of Zea mays, excess Ni causes oxidative damage in the roots of maize seedlings and the capacity of enzymes involved in the detoxification of oxygen free radicals was also investigated [5]. The toxicity of Ni decreased in gas exchange characteristics in many plant species of *Oryza sativa L.*[43] In the case of Mungbean, the accumulation of Ni decreased the rate of growth, photosynthetic pigments and yield attributes[1]. In the case of Fenugreek, the highest accumulation of Ni within roots and very less concentration in stem and leave was observed[44].

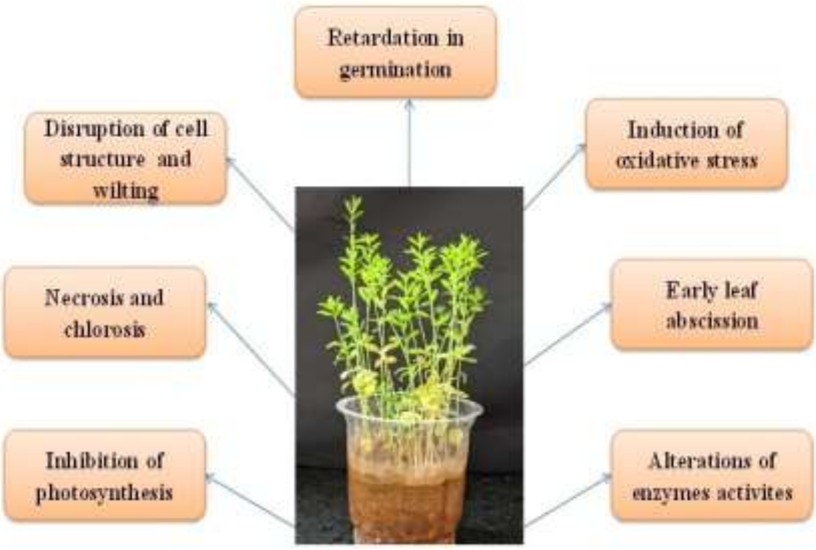

Fig. 4: Nickel stress affects plants

Table 2
Some plant species are affected by Nickel stress

Crop	The toxicity level of Ni	Effects on plants
Guava	1000µM	Progressive decline in the plant growth, substantial K^+ losses
Rice	400µM	Roots and Shoots have a shorter fresh weight and length.
Rice	60µM	Inhibition of the Root growth
Wheat	100µM	Reduction of physiological growth of the plants
Wheat	200µM	Reduction of chlorophyll contents inhibits shoot growth
Wheat	40mmol m^{-3}	Reduction of plant nutrition
Potato	0.3mM	Reduction of chlorophyll, plant growth
Barley	200mM	Reduction of root biomass

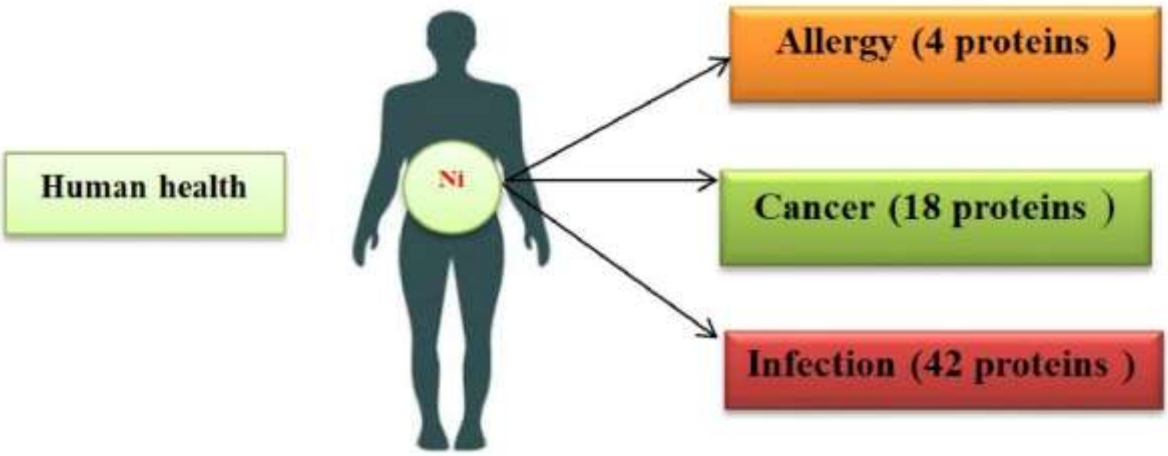

Fig. 5: Nickel (Ni) effect on human health is an intrinsic disorder

Toxic Effect of Nickel on Human health through plants: Although vegetables are the primary source of heavy metals in human diets, heavy metal exposure can cause major health concerns. The toxic effect of heavy metals on human was shown in figure 5. Heavy metals accumulate in green leafy plants without causing poisoning symptoms[28]. A small quantity of nickel (Ni) is essential for human health. The uptake of a higher amount it can be hazardous. Ni is also a micronutrient and essential nutrition for the proper functioning of the human body.

Nickel-containing foods include cocoa and dark chocolates fruits such as almonds, pineapple and plums, grains such as millets, oats, brown rice, sesame seeds and sunflower seeds and vegetables such as broccoli and cauliflower (beans, cabbage, leeks, peas, potato, tomato, green beans and broccoli). Because Ni and its compounds are probably carcinogenic, they have negative impacts on human health such as lung fibrosis, renal and cardiovascular illness and cancer of the respiratory system, as well as a high prevalence of nasal and lung cancer [11].

Sources of Cobalt presence in Environment: Another naturally occurring heavy metal is cobalt (Co) which occurs as smaltite, erythrite and cobaltite in the earth's crusts. Co can be accumulated by plants from the soil. The atomic weight of Co is 58.933 and the atomic number is 27. The melting point is 1768K and the boiling point is 3200K. The oxidation states of Co include Co (II) and Co (III). Naturally, Co occurs in many different chemical forms in our environment. The toxic effect of Co is quite low than the other heavy metal in the environment. Accumulation of Co is of great importance in crops as it contributes toxicity to the human food chain. In acidic soil, the potential for Co toxicity is high at any concentration.

Effect of Cobalt on the plant: The absorption and distribution of Co in plants depend upon the plant species and are controlled by different mechanisms. Cobalt is important for plant growth and development because it regulates water use and reduces transpiration. Many enzymes and co-enzymes require Co [4]. The heavy metal conducts the direct inhibition of an enzymatic reaction in chlorophyll synthesis[55]. A high concentration level of Co decreased the formation of nodules, inhibition in the formation of roots resulting in reduced development, chlorosis and necrosis and crop yield[46]. Cobalt sulfate is more harmful than cobalt chloride.

Cobalt (Co) is one of the beneficial elements [56], which helps at low concentrations. It promotes plant growth and seedpod yield, increases drought resistance and leguminous plants produce nodules, such as faba bean and soybean. Co is an essential component of cobalamin (vitamin B12), which is required for various nitrogen-fixing enzymes to function.

Several enzymes and coenzymes in living beings require cobalt[58].

Co plays a vital role in the hormonal level in the water balance of tomato, high yield and quality of wheat, squash plants, olive, groundnut, faba bean, cucumber, cowpea and sweet potato. At higher concentrations, Co., decreases the activities of catalase and peroxidase and increases auxin and gibberellin, therefore the catabolism is rather than anabolism[10]. Excess amounts of deposition of Co stress in the soil can also disturb the uptake of other essential elements by the plant.

Mostly Co content was found to be higher in roots than shoots because roots are usually more affected by metals as compared to shoot and a small amount of metal translocated to shoot. Co toxicity hampered growth in terms of length, fresh and dry mass of roots, shoots and the leaf's effective area.

The effect of Co stress on growth inhibition is thought to be largely due to suppression of cell elongation during the early stages of toxicity. Co-stressed plants' development and leaf area are affected as they become older. Toxicity also affects water relations and membrane permeability in Co-stressed plants, resulting in a photosynthetic deficiency. The higher concentration of Co-treatment causes reduction in the number of flowers and production of fruits [22].

The toxic concentration of Co inhibits active ion transport and reduces shoot weight and greening in plants. Under the treatment of Co, increasing levels of Co markedly increased the proline content of all the test varieties of soya bean. Accumulation of proline or some other organic solute may be the corresponding solute for maintaining the osmotic balance between the vacuoles and cytoplasm [31]. Co is known to cause irreversible damage to several vital metabolic constituents, plant cell walls and cell membranes. The effect of Co, at high and low concentration, on plant growth physiology is depicted in the figure 6.

Accumulation of Cobalt in plant: At the high level of Co, in the case of *R. sativus* plants, the length of shoot and roots decreased by inhibiting cell division or cell elongation. The amount of nitrogen, phosphorus content of these plants decreased as the concentration of Co level increased in the soil. The amount of sugar content of Radish plants decreased with an increasing level of Co in the soil[24].

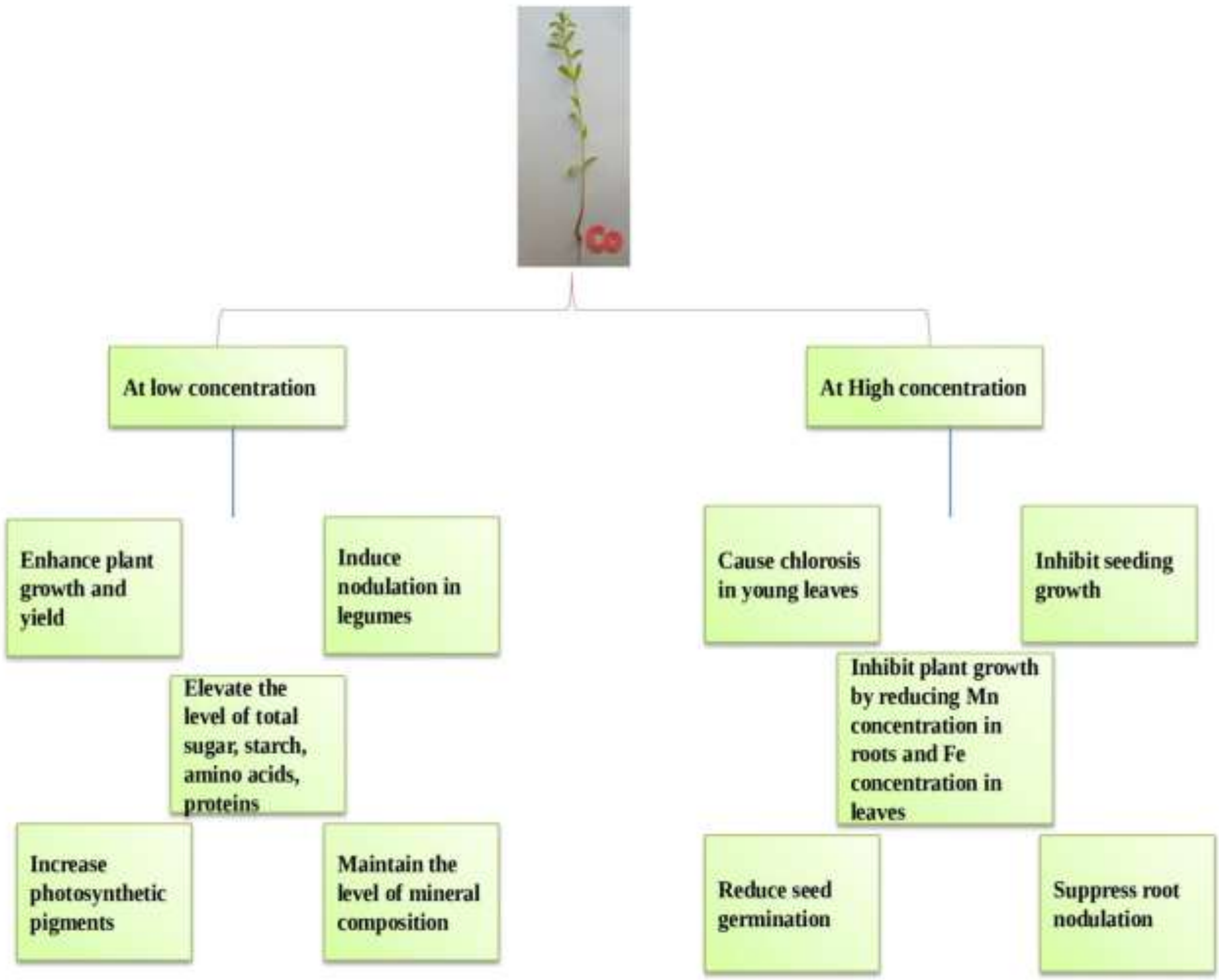

Fig. 6: Effect of Cobalt in plants at low and High Concentration

In legumes plants, Co is essential for the microorganisms for fixing atmospheric nitrogen. In the case of barley, oilseed rape and tomato plants phytotoxicity study reported the adverse effect of Co on shoot growth and biomass. Excessive Co inhibited the concentrations of iron, chlorophyll, protein and catalase activity in cauliflower leaves[12]. In the case of soya bean on the treatment of Co, the maximum Co content of soybean root, nodules, stem and leaves was studied at 250 mg kg^{-1} concentration of Co level in the soil and the minimum Co accumulation in roots was observed in normal (control) plants. The treatment of Co at 50 mg/kg soil was found to be suitable for the overall growth of soya bean plants[23].

In Cowpea the beneficial effects of Co in low concentration at 8 ppm, enhanced the stressed plants' growth and yield and induced modulation. In the case of maize at 50 mg, Co kg^{-1} enhanced the growth of the seedling, photosynthetic pigments, elevated the level of protein content and starch. Co has been undamaging for chickpea. In the case of beans on the treatment of Co, their effects on the activity of amylases and proteases were observed in the germinating stages while the effect on photosynthesis chlorophyll, sugars, carbohydrate and protein content at the seedling stage and the flowering stage was observed[60]. In the case of pea plants, a higher concentration of Co prevents root growth by retarding cell division and by inhibiting the nutrients and water uptake and translocation[46].

In Mungbeans, Co at high concentration (5µM) was reported to inhibit the growth of the seedling, Chlorosis is induced in early leaves, as well as a reduction in Mn and Fe concentrations in roots and leaves, resulting in a reduction in plant growth[4]. In *Trifolium pratense* L, in all cultivars, the application of Co had a beneficial influence on yield component values and seed yield. Increased nodulation and nitrogen fixation were the indirect effects of Co on plants [54].

Toxic Effects of Cobalt on Human health: In the human body, Cobalt is utilized for the absorption and processing of vitamin B_{12}. It is used as an essential element in cell culture media. Besides, cobalt helps in treating various health ailments such as anemia and certain infectious diseases, repairing myelin, which surrounds and protects nerve cells and also helps in the formation of hemoglobin (red blood corpuscles). An excess amount of Co causes heart muscle disease, nausea, vomiting. Decrease in the Co concentration level in blood may cause visual damage which includes optic nerve atrophy, reduced visual acuity, retinal dysfunction, complete blindness, optic nerve atrophy, poor color vision, blurred vision, memory loss, impaired attention, muscle weakness, the decreased function of sensory stimuli, shortness of breath, low thyroid function, lack of appetite, rough hair coat and Neurological disorder[33].

Overdosage of Co in humans causes decrease of fertility in men, abnormally increased Hb in blood. Studies have also reported the treatment of pregnant women with $CoCl_2$ for the elevation of hemoglobin and hematocrit levels without disturbing the thyroid or liver function during pregnancy. The major carrier protein for Co (II) ions in the blood is serum albumin and 2-alpha-macroglobulin and it also binds to lipoproteins and haptoglobin[40].

Conclusion

Sources of cobalt and nickel and their different concentration levels significantly affected the growth, yield and quality of flaxseed. It has been found that contamination of the soil with heavy metals suppresses seed germination. The toxicity produced by nickel and cobalt impaired the growth in terms of length, fresh and dry weight of roots and shoots. Inhibition of root growth is considered to be primarily the result of the inhibition of cell elongation. Heavy metals can also denature chlorophyll pigments and carotenoids.

In the present study, Ni and Co suppressed chlorophyll a, chlorophyll b and carotenoid levels in the leaves. Metal toxicity may have caused leaf tissue tanning and necrosis. The presence of Ni in the plant is more hazardous for living organisms, but Co in the plant is helpful for the living organism because cyanocobalamin (Vitamin B12) contains Co which is valuable for us. However, excess amount of Co is also hazardous to the living organism.

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