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Accumulation and Translocation of Nickel and Cobalt in Nutritionally important Indian vegetables grown in artificially contaminated soil of Mumbai, India

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Abstract

The present investigation deals with assessment of translocation and accumulation of the two heavy metals nickel and cobalt from artificially contaminated soil to nutritionally significant Indian vegetables. The concentration was determined in different plant parts and subsequently the transfer factor and translocation factor were determined. The study presented that metal concentrations in plant tissues and soil were in association with each other. Thus at higher metal concentration in soil, the metal concentration may exceed permissible levels causing toxicity in plants as well as its successive components of food chain. None of the three species were detected to be hyperaccumulators. Maximum metal concentration was detected in root followed by stem and then in leaf. This it can be stated that leaves which are of dietary importance absorb relatively lower levels of metal than roots.

Keywords: Heavy metals, bioaccumulation, toxicity, phytoremediation, nickel, cobalt.

Introduction

Urbanization and industrialization have resulted in pollution of soils. The effects of this soil contamination can be observed prominently in the urban environments of developing nations like that of India[1]. Growing demand for food as a result of increasing population has led to a substantial increase in the application of agro chemicals like fungicides, insecticides, herbicides, and fertilizers[2]. Heavy metals are an integral component of these substances. Srivastava and Singh (2012) also stated that atmospheric pollution has an ability to alter soil pH[3]. Heavy metal pollution of soils is of serious concern as they are persistent, non-biodegradable and become toxic to living organisms[4]. They occur in various soluble and particulate materials which also affect their bioavailability and mobility[5]. They have a tendency to accumulate in soft tissues of living organisms. The deposition may then show biochemical or physiological changes in them[6]. Extreme values may cause growth inhibition and loss in net production, prominently seen in plants[7].

The current study focusses on th estudy of accumulation of two metals – Nickel (Ni) and Cobalt (Co) within nutritionally important Indian plant varieties. The selection of these two metals is not concerned with their biogeochemical properties. Selection was done on the basis of their potential in causing threats to various biotic and abiotic components of environment[8]. These metals have the capacity to cause acute as well as chronic toxicity. It not only disturbs the growth, development and maturity of plants and animals but also human health.

Many plant species as a whole are important sources of minerals and vitamins[9]. Plants exhibit an inherent ability to absorb toxic substances including heavy metals along with nutrient materials from soil. Hence such non-essential toxic metals are found within plant tissues causing toxicity[10]. These materials are then accumulated in its tissues and subsequently transferred to higher organisms along the food chain[11]. While this property of phytoremediation is extremely useful for clean-up of contaminated soils, it may turn toxic to higher organisms of food chain, especially humans, if the plants are food crops. Toxic heavy metal loaded agricultural soils have now emerged as a threat.Metal concentration may increase to such an extent, that it may cause toxicity to crop plants[12].

Material and Methods

Current study includes assessment of accumulation of Ni and Co within root, stem and leaves of - Spinach (*Spinacia oleracea*), Red Amaranth (*Amaranthus cruentus*) and Fenugreek (*Trigonella foenum-graecum*). The leaves of these 3 plants are consumed for their nutritional importance. The seeds of these three plants were obtained from Namdeo Umaji Agritech, India Private Limited, Mumbai. The seeds were washed thoroughly in deionised distilled water. Seeds were then germinated.

New and clean plastic pots were selected. (Dimension: length × breadth × height = 50cm × 30cm × 40cm). Plastic pots give abenefit of removal of possible source of contamination that may arise from soil or cement pots. They are also light weight and leakage proof. The pot size was selected in such a way that the depth is more than maximum plant root length and area is sufficient for generation of adequate quantities of sample. Soil from a nursery in Tarapur, district Thane, Maharashtra, India was brought in to laboratory. Dirt, pebbles, stones, leaves, sticks, etc. were removed. At least 10Kg of fine textured soil was then filled in the pots.

The soil was contaminated with $Ni(NO_3)_2$ and $Co(NO_3)_2$ (20mg salt per Kg of soil) as solution in distilled water. The two metals were selected because of their least reported synergistic and antagonistic effects. AR grade chemicals from Burgoyne & Co., Mumbai India were used. The soil was homogenized thoroughly for uniform distribution of metals. Half Kg of vermin-compost was then added. Chemical fertilizers and pesticides were not used. Such soil was kept steady for 1 week for stabilization. Sprouted seeds were later on planted in each pot and allowed to grow up to harvesting time (3 pots per plant species). The pots were watered every day morning using distilled water in order to avoid contamination. The pots were kept in ambient sunlight but under shade. They were protected from rain water. It took approximately 60 days for the plants to be ready for harvest. A single pot as control was maintained separately[13-15].

Sample collection: Plant samples were harvested 60 days after sprouting. Since the metal content of plant varies with plant age and stage of maturity, a harvesting period of 60 days was kept fixed. Soil was also sampled from the same place where plant has been sampled[16]. Plant samples were collected carefully using a clean plastic hand trowel to dig the soil and were gently removed, so that no plant part is lost. Such samples were temporarily stored in self-sealing and labelled plastic bags. Plant samples were initially washed thoroughly using tap water and later with distilled water to clean them off contaminants. The leaves were then separated using a clean steel knife. Soil samples were screened for pebbles, dirt, sticks, etc. Such soil was then dried in oven at 105ºC for complete moisture removal. It was then passed through 20mm sieve using a magnetic sieve shaker to collect fine soil necessary for further processing[15].

Digestion and determination: 2g. of oven fried vegetation sample was kept in muffle furnace at 400ºC to convert it into ash. It was then digested using a mixture of HNO_3 and $HClO_4$ in the ratio 5:1. Digestion was performed externally on a hot plate. It was continued till a minimal clear layer of acid was obtained. After cooling, this content was filtered through Whatman filter paper number 41. Final volume of 25ml was made in a clean volumetric flask with 0.25% HNO_3.

Soil digestion was done in Microwave digestion system (Ethos I Advanced unit). 2g. of oven dried soil was taken in Teflon containers and digested with a mixture of HNO_3: HF in the ratio 8:1. The digested content was centrifuged and filtered. Final volume of 25ml was made using 0.25% HNO_3.

Each sample of soil and vegetation was digested in replicate for consistency of results. The analysis of Ni and Co was done using AAS. Each sample was analysed twice to obtain typical results and the data reported in mg/Kg.

Results and Discussion

Physicochemical properties of soil: Concentration of free metal ion depends on both total concentration of metal soil as well as a number of other factors[16]. The soil texture was sandy loam. Soil pH is an important governing factor. It is affected by the reaction of atmospheric CO_2 as well as organic and inorganic materials around[17]. Average pH was 7.26 (slightly alkaline). The soil pH was as per necessity for appropriate growth and efficient uptake of nutrient materials from soil[18]. Organic matter content of soil was determined to be 1.9 g%. The soil CEC was calculated to be 40.2Cmol/Kg. The initial nickel concentration of uncontaminated soil was determined to be 16.67 ± 0.07 mg/Kg and the same for cobalt was detected to be 18.25 ± 0.8 mg/Kg. The Ni and Co concentration of vermin-compost and seeds were below minimum detectable level.

Concentration of Ni and Co in plant and soil samples: Results of assessment of Ni and Co in parts of plants are outcome of soil contamination and later, the content of such toxic materials in vegetation including field crops. As per the heavy metal content, high requirements are posed especially on productive parts of plants that are used in human nutrition as plant products, raw-materials of food industry, forage crops, from which heavy metals could be transferred into different products[4].

The evaluation of contaminating heavy metals is a serious problem and is expanding. According to Food Codex heavy metals as pollutants can translocate into food materials from soil (via roots) and air (mostly via leaves), in some cases also from equipment, etc[4,19]. Soil acts as a sink of heavy metals, from which they are transferred into plant products and through food chain into animal products. Current study showed that the concentration of both Ni and Co was detected maximum in soil. The mean concentrations of Ni and Co in soil were detected to be 49.59±0.06mg/Kg and 54.19±1.06mg/Kg respectively in comparison with the background concentrations as 16.67± 0.07 mg/Kg and 18.25±0.8 mg/Kg respectively. The metal concentration in soil was thus detected to be far more than concentration in plant parts. Similar observations were made by Nwajei G.E et al during their study on tomato fruits and leaves[20].

Metal concentration in soil is a dominant factor in soil to plant transfer of heavy metals[21]. Availability of heavy metals may also be directly influenced by plant specificity itself[22]. Peak concentration of these heavy metals was recorded in roots of the three plants followed by stem and then in leaves, except Ni concentration in spinach. Ni concentration of Spinach was found to be the highest in roots followed by leaf and then in stem. This indicates that the three plants show better retention of metals in roots itself. Plant roots act as a barrier, thereby avoiding free flow of materials from soil to shoot parts. A similar pattern was demonstrated by Jaykumar and Jaleel while working on cobalt in their experiments on Soyabean[23]. Plant specific interaction of Ni with spinach however, shows higher translocation of metal in leaf than stem.

Concentration of Ni and Co in roots of three plants: Average concentration of Ni in Spinach roots showed concentration of 2.93±0.02mg/Kg. The same for Fenugreek and Red Amaranth were detected to be 1.93±0.07mg/Kg and 3.92±0.05mg/Kg respectively. Concentration of Ni in control was found out to be 0.98±0.05mg/Kg, 0.75±0.04mg/Kg and 0.84±0.06mg/Kg respectively for Spinach, Fenugreek and Red Amaranth. The results hence show a 3 to 4 times rise in metal concentration in roots tissue of sample. The figures indicate that roots of Red Amaranth are a better accumulator of Ni, followed by Spinach while the least absorption was shown by Fenugreek.

For Co, the metal concentration in Spinach, Fenugreek and Red Amaranth were found out to be 1.47±0.06 mg/Kg, 1.19±0.05 mg/Kg and 1.73±0.04 mg/Kg respectively. The same in control experiments were detected to be 0.49±0.03 mg/Kg, 0.53±0.06 mg/Kg and 0.72±0.06 mg/Kg respectively. The values show that Co is absorbed in higher concentrations by Red Amaranth followed by Spinach while the least by Fenugreek.

Hence in case of plant roots, for both the metals, maximum absorption was reported by Red Amaranth while the least by Fenugreek, Spinach concentration remained in between the two. A collective conclusion can be drawn here that the roots of three plant species show a higher affinity towards Ni than Co.

Concentration of Ni and Co in stem of three plants: Plant stems are a necessary part of the anatomy as they carry water and nutrient materials from soil up to the leaves. Through an intense network of xylem and phloem, the materials are carried up. Thus stem acts as a transient connection. Since the stem of herbaceous leafy vegetables is tender, they also serve for nutritional importance. The average Ni concentration in stem of Spinach, Fenugreek and Red Amaranth was detected to be 1.85±0.05 mg/Kg, 0.81±0.05 mg/Kg and 1.21±0.04 mg/Kg respectively. Control experiments for the three plants showed Ni concentration as 0.19±0.02 mg/Kg, 0.14±0.08 mg/Kg and 0.13±0.04 mg/Kg respectively. Thus Spinach showed a higher tendency to accumulate Ni in its stem followed by Red Amaranth, Fenugreek being the least.

Co concentration in the three plant varieties Spinach, Fenugreek and Red Amaranth was detected to be 0.83±0.04 mg/Kg, 0.59±0.04 mg/Kg and 0.33±0.03 mg/Kg respectively. The subsequent Co concentration in control experiments was detected to be 0.49±0.03 mg/Kg, 0.16±0.06 mg/Kg and 0.23±0.05 mg/Kg respectively. The values showed that Spinach has a higher tendency to accumulate Co in its stem followed by Red Amaranth and the least by Fenugreek, when the soil concentrations are less. At higher concentrations, Fenugreek accumulates higher quantities of Co as compared with Red Amaranth in their stem, Spinach remains the highest accumulator. Since Spinach stems have dietary significance, Co contamination should be studied carefully and to be given prime importance.

Table 1 and table 2 respectively show the concentration of Ni and Co in soil before (control) and after contamination and the concentration of the metal uptake in root, stem and leaves of the three vegetable species, Spinach, Fenugreek and Red Amaranth.

Concentration of Ni and Co in leaf of three plants: Due to their dietary significance, leaves are given utmost attention. In comparison with roots, the leaves of all plant species exhibited lesser concentration. The same tendency was observed in control experiments also. Thus the absorption pattern did not change at elevated metal concentration in soil.

Table-1
Concentration of Nickel (Ni) in morphological plant parts of Spinach, Fenugreek and Red Amaranth and corresponding soil samples

Sample		Spinach			Fenugreek			Red Amaranth		
	Soil	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf
Set I	48.27± 0.06	2.98± 0.01	2.51± 0.08	1.84± 0.04	1.97± 0.09	0.70± 0.03	0.42± 0.06	3.69± 0.03	1.11± 0.04	0.96± 0.02
Set II	49.97± 0.08	2.87± 0.03	1.45± 0.03	2.18± 0.04	1.86± 0.07	0.78± 0.05	0.36± 0.04	3.96± 0.07	1.29± 0.05	1.12± 0.06
Set III	50.52± 0.04	2.93± 0.03	1.60± 0.04	2.30± 0.05	1.95± 0.06	0.96± 0.06	0.25± 0.06	4.11± 0.05	1.23± 0.04	1.50± 0.05
Avg. for samples	49.59± 0.06	2.93± 0.02	1.85± 0.05	2.11± 0.04	1.93± 0.07	0.81± 0.05	0.34± 0.05	3.92± 0.05	1.21± 0.04	1.19± 0.04
Control	16.67± 0.07	0.98± 0.05	0.19± 0.02	0.17± 0.06	0.75± 0.04	0.14± 0.08	0.16± 0.03	0.84± 0.06	0.13± 0.04	0.09± 0.03

Table-2
Concentration ofCobalt (Co) in morphological plant parts of Spinach, Fenugreek and Red Amaranth and corresponding soil samples

Sample		Spinach			Fenugreek			Red Amaranth		
	SoilC (mg/Kg)	Root (mg/Kg)	Stem (mg/Kg)	Leaf (mg/Kg)	Root (mg/Kg)	Stem (mg/Kg)	Leaf (mg/Kg)	Root (mg/Kg)	Stem (mg/Kg)	Leaf (mg/Kg)
Set I	52.37± 1.1	1.312± 0.5	0.63± 0.04	0.49± 0.03	0.98± 0.04	0.55± 0.03	0.36± 0.02	2.02± 0.03	0.52± 0.06	0.25± 0.03
Set II	54.94± 0.9	1.519± 0.9	0.87± 0.03	0.371± 0.06	1.355± 0.06	0.61± 0.05	0.498± 0.06	1.46± 0.05	0.601± 0.04	0.246± 0.03
Set III	55.26± 1.2	1.589± 0.6	0.994± 0.06	0.619± 0.03	1.232± 0.04	0.598± 0.05	0.622± 0.08	1.699± 0.05	0.721± 0.03	0.493± 0.05
Avg. for samples	54.19± 1.06	1.47± 0.06	0.83± 0.04	0.49± 0.04	1.19± 0.05	0.59± 0.04	0.49± 0.05	1.73± 0.04	0.61± 0.04	0.33± 0.03
Control	18.25± 0.8	0.49± 0.03	0.13± 0.04	0.13± 0.02	0.53± 0.06	0.16± 0.06	0.15± 0.02	0.72± 0.06	0.23± 0.05	0.14± 0.05

The leaves of Spinach showed highest Ni concentration (2.11±0.04 mg/Kg) trailed by Red Amaranth (1.19±0.04 mg/Kg) and the least by Fenugreek (0.34±0.05 mg/Kg). Thus Spinach is a better absorber of nickel but Fenugreek, with the least metal uptake; is suitable for consumption. Concentration of Co was reported approximately equal in Spinach (0.49±0.04 mg/Kg) as well as Fenugreek leaves (0.49±0.05 mg/Kg). Co concentration in Red Amaranth was seen to be the least (0.33±0.03 mg/Kg). The results can thus be interpreted as among the three plants studied Spinach and Fenugreek show similar tendency to accumulate Co in its leaf tissues but considering dietary importance Red Amaranth is better than others.

Somewhat similar results were found by Jaykumar and Jaleel while working on Soybean with cobalt as the target metal[23]. The concentration in roots was detected to be the maximum followed by stem and then in leaves. Farago and Mahmoud had studied extensively on heavy metals. They indicated that Ni and Co behave differently in soil and plant tissues. The two metals are actively absorbed by roots of the plant and is evenly distributed in other plant tissues with considerable accumulation in tops. Similar results were reported in current study too[24].

Transfer Factor (TF): Metals from soil are absorbed by plant roots and then distributed in various plant tissues. Such transmission of metals from soil to plant tissues is studied using an index called as Transfer Factor (TF).It is calculated as a ratio of concentration of a specific metal in plant tissue to the concentration of same metal in soil, both represented in same units[15]. Higher TF values (≥1) indicate higher absorption of metal from soil by the plant and higher suitability of the plant for phyto-extraction and phytoremediation[25]. On the contrary, lower values indicate poor response of plants towards metal absorption and the plant can be used for consumption.

Table 3 gives a list of TF values for both Ni and Co in the three plant varieties. The current study showed that the TF values varied between 0.006 and 0.007 for Ni and 0.006 to 0.03 for Co. All the values reported here were found to be very well less than 1 indicating that the three species of plants are not suitable for clean-up of heavily pollutedsoil through phytoremediation. In Ni, TF value was detected to be highest in roots while the least in stem. Leaves showed intermediate TF values. In case of Co, roots of all the three plants indicated relatively higher TF. Thus it can be concluded here that, the roots of the three plant species that are nutritionally insignificant to humans are absorbing higher concentration of Ni and Co. Whereas leaves of these plants that are of highest dietary importance are absorbing relatively lesser concentration of metals. The TF values for Ni were found to be higher than Co indicating that the three plants show higher affinity towards Ni than Co. Overall TF values are thus found to be significant.

Translocation Factor (TrF): The gradual movement of solute (metal ions) from soil to plant roots and from roots via stem to plant leaves is referred as translocation of solute materials. It is determined as the ratio of metal concentration in shoot to its concentration in the root tissues[26]. Translocation Factors (TrF) of the three plant species Spinach, Fenugreek and Red Amaranth were evaluated for both metals, Ni and Co. The results have been mentioned in table 4. TrF values nearer to zero, indicate increased retention of metals in plant roots with very less movement to above soil plant parts[14].

The results indicate that Spinach showed highest TrF value for Ni while the least TrF for Ni was given by Fenugreek. The value for Spinach shows that its roots are not holding higher quantities of Ni but the same is sent to shoot parts that are of much dietary importance. In case of Fenugreek, Ni is retained within roots itself with very less concentration reaching stem and leaf collectively. This shows the safety of Fenugreek for consumption. The TrF of Co for Spinach and Fenugreek were detected to be similar. TrF for Spinach was found out to be 0.89 while the same for Fenugreek was seen to be 0.907. The values were found to be approaching 1 indicating higher translocation of metal from root to shoot parts. The TrF value for Red Amaranth was found to be moderate (0.543). Similar results were found by Duman and Ozturk (2010) while studying on Ni contaminated soil[27].

Table-3
Transfer factor (TF) values of plant tissues of three plant species for metals Ni and Co

Sr. No.	Vegetation type	Plant Tissue	Avg. concentration in vegetation		Transfer Factor (TF)	
			Ni (mg/Kg)	Co (mg/Kg)	Ni	Co
1	Spinach	Root	2.93±0.02	1.47±0.06	0.05	0.02
2		Stem	1.85±0.05	0.83±0.04	0.03	0.01
3		Leaf	2.11±0.04	0.49±0.04	0.04	0.009
4	Fenugreek	Root	1.93±0.07	1.19±0.05	0.03	0.02
5		Stem	0.81±0.05	0.59±0.04	0.01	0.01
6		Leaf	0.34±0.05	0.49±0.05	0.006	0.009
7	Red Amaranth	Root	3.92±0.05	1.73±0.04	0.07	0.03
8		Stem	1.21±0.04	0.61±0.04	0.02	0.01
9		Leaf	1.19±0.04	0.33±0.03	0.02	0.006

Note: Conc. in soil: Ni = 49.59±0.06mg/Kg and Co = 54.19±1.06mg/Kg, All values reported in dry by dry basis

Table-4
Root to shoot translocation factors (TrF) for three plant species and two metals Ni and Co

Sr. No.	Vegetation type	Concentration in aerial plant part		Concentration in root		Translocation Factor (TrF)	
		Ni (mg/Kg)	Co (mg/Kg)	Ni (mg/Kg)	Co (mg/Kg)	Ni	Co
		(a)	(b)	(c)	(d)	(a/c)	(b/d)
1	Spinach	3.96±0.09	1.32±0.08	2.93±0.02	1.47±0.06	1.35	0.89
2	Fenugreek	1.15±0.1	1.08±0.09	1.93±0.07	1.19±0.05	0.59	0.907
3	Red Amaranth	2.4±0.08	0.94±0.07	3.92±0.05	1.73±0.04	0.61	0.543

Note: All TF values reported in dry / dry basis

The values in table 4 propose that Spinach has lower retention of both Ni and Co in its root tissues. Both the metals are actively transported to the shoot parts. Similar conclusion was given by Farago and Mahmoud[24]. The other two plants were found to be moderate in translocation of metals.

Prevention of Food Adulteration Act, 1955, Government of India have categorised nickel as a poisonous metal. The various food materials should not contain the level of Ni more than 1.5mg/Kg[28]. The FAO as well as WHO[29] have prescribed a limit of 0.2 mg/Kg for Ni. The concentration of Ni in control experiments was found to be well within the prescribed limits. But at higher concentration of Ni in soil, it was seen that the plants tend to accumulate higher quantities of the metal within its tissues. Thus all the three plant parts of all three species showed a tendency to accumulate higher levels of Ni when high Ni concentration was provided in soil. Ni concentration in samples was found to exceed multiple times than the given permissible limit except for the stem and leaf of Fenugreek. Thus it can be said that, the consumption of Spinach and Red Amaranth grown in Ni contaminated soil needs to be avoided. Fenugreek leaves, may however be consumed. Further study needs to be done with more number of samples to come to a more uniform result. Indian standards do not specify permissible limits for cobalt.

Conclusion

The nickel and cobaltconcentration of the three nutritionally important plant varieties namely Spinach (*Spinacia oleracea*), Fenugreek (*Trigonella foenum-graecum*) and Red Amaranth (*Amaranthus cruentus*) with corresponding soil concentration were determined in artificially contaminated soil. Study was significant because of the dietary importance of the three target vegetables. Subsequently, the transfer factors (TF) and translocation factors (TrF) were determined in order to draw conclusions. It was seen that both the metals were absorbed readily by all the three plant varieties. The three plants showed higher affinity towards Ni than Co in absorption. However, TF values did not approach 1. More specifically the values were found closer to 0. This proved that all the three plants are not hyper-accumulator species and cannot be used for clean-up applications. However, root to shoot transfer was moderate. It was very high for Ni in Spinach. The comparison with international as well as Indian permissible standards shows that soil metal concentration remains in correlation with plant metal concentration. At higher levels of soil metal concentration, plant metal concentration may exceed permissible limits causing toxicity in living organisms.

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