*Pol. J. Environ. Stud. Vol. 34, No. 1 (2025), 573-592*
DOI: *10.15244/pjoes/186577* ONLINE PUBLICATION DATE: 2024-06-28

*Original Research*

# Influence of Trace Elements (Co, Ni, Se) on Growth, Nodulation and Yield of Lentil

**Muhammad Abbas Anees[1], Syed Ijaz Ul Haq[2], Muhammad Rasheed[1], Mujahid Hussain[2, 3], Ghulam Qadir[1], Rifat Hayat[1], Muhammad Arshad Ullah[4], Abeer Hashem[5], Elsayed Fathi Abd Allah[6], Yubin Lan[2*]**

[1]Pir Mehr Ali Shah Arid Agriculture University, Murree Road, Shamsabad, Rawalpindi-46300 Punjab, Pakistan
[2]School of Agriculture Engineering and Food Sciences, Shandong University of Technology, Zibo, China, 255000
[3]Shandong Agriculture and Engineering University, 255300, Zibo, Shandong.
[4]Pakistan Agricultural Research Council, Islamabad, Pakistan
[5]Botany and Microbiology Department, College of Science, King Saud University, P.O. Box. 2460, Riyadh 11451, Saudi Arabia
[6]Plant Production Department, College of Food and Agricultural Sciences, King Saud University, P.O. Box. 2460, Riyadh 11451, Saudi Arabia

*Received: 21 November 2023*
*Accepted: 27 March 2024*

## Abstract

Lentil (*Lens culinaris* Medic) is the main pulse crop and usually has low production due to numerous factors and cultivation practices. A field study was designed to investigate the best trace elements (Co, Nin, and Se) for sole application or in combination as fertigation after sowing for the lentil variety (NIA Masoor-2005). Comparatively better crop growth and yield with better quality were obtained through the application of Co, Ni, and Se, at 600, 600, and 300 g $ha^{-1}$, respectively. The maximum grain yield (1638 kg $ha^{-1}$) was harvested from the treatment where Co, Ni, and Se were applied at 600 g $ha^{-1,}$ which was 15% higher than that of the control receiving no trace elements. According to economic analyses, the best performing treatment is the use of Ni and Co in full doses, as Se has high and does not have economically effective results. All three trace elements had a positive impact on lentil nodulation (up to 33%), seed protein (4.6%), and yield increase (15%) over the control receiving no trace elements. However, with the application of Co and Ni at 600 g $ha^{-1}$, each was found to be the most economical and showed comparable results regarding growth, nodulation, and yield contributing parameters compared to Co, Ni, and Se, each at 600 g $ha^{-1}$. It was concluded that the use of trace elements (Co, Ni, and Se), individually or in combination, is economical and has the capacity to increase the yield and nodulation of lentils.

**Keywords:** economics, nodulation, pulses, Rhizobium inoculation, trace elements

*e-mail: ylan@sdut.edu.cn

## Introduction

Lentil (*Lens culinaris Medic*) is a main crop usually grown in the rain-fed area of Pakistan. Crops were grown in an area of 10.4 thousand hectares from 2015-16 in Pakistan, with a total production of 6.0 thousand tons (Government of Pakistan, 2018-19). Lentil production had a secondary position among the cereal crops in Pakistan. In Pakistan, lentil is the second most important food legume in the winter season, following chickpea. Pakistan contributes 8% of the total lentils produced in the world. There are several reasons for imbalanced lentil yields, mostly in the Pothwar region of Pakistan. Scarcity of water, low soil fertility status, and mismanagement are the major burning issues. If grown through some suitable agro-techniques, its yield could be doubled compared to the present one, and it would increase farmers' income significantly [1]. Lentil is a high-priced crop and thus can give more profits to the poor farmers in marginal areas of Pakistan. Lentil is a leguminous crop that biologically fixes atmospheric di-nitrogen ($N_2$). It utilizes nodules to facilitate the fixation of nitrogen in the soil [2]. Biologically fixed nitrogen is used as a fertilizer with a slow release of plant nutrients, resulting in the uniform availability of nutrients up to crop maturity. It is cultivated throughout Pakistan. Lentil is a very important pulse grain, containing 24% protein, and is a high-priced crop mainly grown in irrigated and rain-fed areas. Imbalanced use of nitrogenous-based fertilizers in crop production is common. In Pothwar, resources are poor and farmers sow lentils by harvesting poor yields that are far less than the potential of the current varieties. Pothwar region soils are moisture and nutrient deficient due to low and erratic rainfalls and little use of nutrients, especially trace elements, which decline the yields of rainfed lentils.

Lentil has rich resources of proteins, vitamins, and minerals for human sustenance, whereas straw is a precious animal feed. Furthermore, lentils can facilitate restoring soil fertility and secure their own nitrogen from the atmosphere. The host plant and plant growth-promoting rhizobacteria (PGPR) are familiar with the symbiotic relationship. Keeping this in mind, this study is designed for the isolation and characterization of PGPR from the rhizosphere of lentil and examines the trace element effects on the nodulation of lentil and their consequences on the symbiotic efficiency of Rhizobium leguminosarum along with the yield of lentil. The symbiotic association found among legumes and Rhizobia helps in the configuration of nodules, which are the sites for nitrogen (N) fixation present on the legume's roots [3]. To improve nodulation and N-fixation, seed inoculation of pulse crops with effective Rhizobium strains is a known practice that leads to improved growth and grain yield. Various soil bacteria recognized as PGPR are capable of exerting advantageous impacts on plants and can enhance the yield of an extensive crop. Through various mechanisms, PGPR is capable of promoting growth like production of symbiotic nitrogen fixation, phytohormones, solubilization of mineral phosphates and supplementary nutrients, and aggression by pathogens via the assembly of antibiotics and the decrease of plant ethylene in roots. In the rhizosphere strains, PGPR stimulates one or more of these mechanisms to achieve the maximum yield, and the inoculation of these beneficial microorganisms requires best supervision practices. It has been distinct that crop yield can be limited by the provision of an ordinary physical environment and the inherent genetic perspective of the crop.

Cobalt is a supporter of soil microflora. It can barely be tested in soil but plays a vital role in preserving Rhizobium and other soil bacteria. 0.10 ppm is well thought out to be ideal. It is principally unobserved in soil fertility programs. It is occupied by the fixation of atmospheric N by Rhizobium bacteria. It also promotes a variety of beneficial soil bacteria.

Nickel is an essential nutrient for the growth of some microorganisms and plants. Nitrogen fixation can be increased with its application. [4] observed that the application of Ni to the soil for the cultivation of soybeans improved the yield and nodulation weight. In different legume crops, the application of a small concentration of Ni is considered necessary for hydrogenase activation and the growth of root nodules. Nickel is a crucial component of the enzyme urease and has a vital role in nitrogen (N) and the metabolism of urea in higher plants. It was also observed that legumes initially increased their growth and yield. However, with a slight increase in nickel concentration, the yield decreased [5].

Although selenium is toxic in large amounts, selenium is an important nutrient for bacteria and plants. Selenium (Se) is not classified as a necessary higher plant element, while at minimum concentrations, it is an important micronutrient for plants and animals [6]. Selenium counteracts abiotic stresses in plants caused by low temperatures, stress, saline conditions, and heavy metals through the regulation of reactive oxygen species (ROS), antioxidants, chloroplast structures, and the recovery of the photosynthetic mechanism [7]. In China, in an experiment, foliar applications of Se significantly improved Se in legumes at reduced costs compared with soil Se application [8, 9], especially in lentils [10].

The yield of lentils is very low because there is no proper nodulation in plants; rhizobium multiplication is not in the soil; there is no incorporation of trace elements (Co, Ni, and Se); and there is no fertilizer recommendation, particularly trace elements. There has been no work on trace elements for improving nodulation in Pakistan to date; trace elements (Co, Ni, and Se) have been found to be useful for the development of nodules in leguminous plants because both bacteria and plants need these trace elements for their proper growth.

Proper growth of Rhizobium requires macro, micro, and trace elements (Co, Ni, and Se). Poor availability of nutrients is observed in our alkaline, calcareous soil. Nodulation in lentils is poor despite macro- and micronutrient fertilization. However, trace elements (Co, Ni, and Se) have not been tested for improving nodulation in lentils. However, nodulation in other leguminous crops has been realized. These trace elements (Co, Ni, and Se) have never been used in combination to improve the nodulation and yield of the crop. Enhancement of bacterial growth and population boosts nodulation, which ultimately increases crop production.

As far as concern, in Pakistan, limited work has been done on trace elements until now. Soils are deficient in nutrients due to the monocropping system, which wipes all the essential nutrients from the soil and renders it unfertile for the cultivation of crops. Keeping in view all this information and the fact that there is published literature, there is a need to explore the potential of trace elements (Co, Ni, and Se) for improving nodulation under the agro-ecological conditions of the Pothwar. Therefore, an experiment was carried out to assess the response of Rhizobium inoculation, appraise the effects of trace elements (Co, Ni, and Se) on the nodulation efficiency of lentils, evaluate the effects of various combinations of trace elements (Co, Ni, and Se) on growth and yield, and determine the economics of trace element (Co, Ni, and Se) application on lentil crops.

It is stated that the application of micronutrients increases the postharvest soil's uptake of micronutrients and increases nodule formation [5, 11, 12]. Well known researchers and scientists have also stated that the cumulative use of different micro and macronutrients clearly increases the yield of seeds by 55-60% of which 20-25% are contributed by micronutrients [5, 13, 14]. [15] The study revealed that the combined application of zinc, boron, and molybdenum significantly enhanced chickpea yields [16]. [17] noted that deficiencies of zinc (Zn), boron (B), molybdenum (Mo), and Mg are important for plants in many Bangladeshi and Pakistani soils and contribute to subpar crop yields. Previous research in Bangladesh has documented the positive impacts of these micronutrients on groundnut, soybean, chickpea, and mungbean production. However, their influence on lentil cultivation remains to be fully elucidated [18].

## Materials and Methods

### Study Area and Research Design

Two consecutive years (2017-18 and 2018-19) of field studies were carried out to investigate the best trace elements (Co, Ni, and Se), for sole application or in combination for lentils. The lentil variety (NIA Masoor-2005) was sown with a single row hand drill, and trace elements were applied alone and in combination with fertigation after sowing the lentil crop. Field experiments were conducted at the PMAS-Arid Agriculture University research farm area, which is rainfed as shown in (Fig. 1), and the temperature at sowing time is 24C'/75. F. A field study was conducted comprising an RCBD (randomized complete block design) with three sets of replicas for two years; 12 treatments of trace elements were applied in field conditions to measure their effect on lentil crop (NIA-Masoor 2005) attributes such as growth, nodulation, yield, and quality. Each plot size was maintained at 4.8 $m^2$. The NIA-Masoor 2005 lentil variety was sown by using a single row hand drill, and trace elements were applied after sowing the lentil crop [2, 19].

### Field Parameters

The soil samples collected from the research form topsoil (0-5 cm depth) [20] which is selected at the start of research studies, samples collected from each point, labeled, and brought to the laboratory. Lastly, all the soil

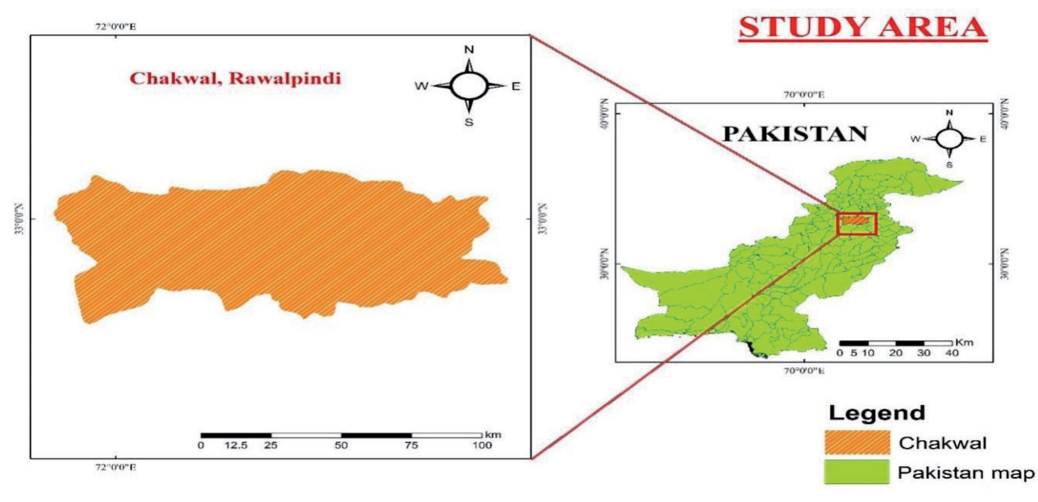


Fig. 1. Field location of the experiment at Knoot Farm, Chakwal.

samples were collected before sowing to determine soil pH and available nutrients [21]. The treatments were $T_1$ (control), $T_2$ (Co 0, Ni600, Se600), $T_3$ (Co300, Ni300, Se300), $T_4$ (Co300, Ni600, Se300), $T_5$ (Co300, Ni600, Se600), $T_6$ (Co600, Ni0, Se600), $T_7$ (Co600, Ni300, Se300), $T_8$ (Co600, Ni300, Se300), $T_9$ (Co600, Ni300, Se600), $T_{10}$ (Co600, Ni600, Se0), $T_{11}$ (Co600, Ni600, Se300) and T12 (Co600, Ni600, Se600). The doses are in gha$^{-1}$.

## Plant Parameters

The height of five randomly selected lentil plants was measured with a common measuring scale from each plot at the stage of harvesting, after which the average height was also calculated in cm. Branches of five plants were counted from each plot at the harvesting stage, and then averages were calculated in plant$^{-1}$. The pod number of five randomly selected plants was calculated from each plot at the harvesting stage in plant$^{-1}$. Pods taken from five plants were dried for eight days. One thousand seeds were separated at random and weighed with an electric balance in the laboratory. The weight of dry shoot parts of lentil plants was randomly taken from five plants in pots at the harvest stage and the average weight was also calculated in plant$^{-1}$ (g).

The root dry weights of five randomly selected plants were recorded in each plot at the harvest stage and then averaged in plant$^{-1}$ (mg). Dry matter was examined on the 75$^{th}$ day after sowing by taking five plants randomly from every plot. Every plant was chaffed, thoroughly mixed, and then oven dried for ten days. Then, the dry matter yield per hectare was calculated (kg ha$^{-1}$). Plants were taken from a one-meter square area, pods were removed, and after drying, the grain yield per plot was calculated and then converted into grain yield. Plants were taken from a one-meter square area of each plot and sun dried for ten days, after which they were weighed using a common measuring balance (kg ha$^{-1}$).

The leaf area of the five selected plants at random from each plot was calculated manually after the average leaf area per plant was taken (cm$^2$). The leaf area index (LAI) was derived from the formula LA = leaf area/land area. The crop growth rate (CGR) was determined using the formula in [22].

$$CGR = \frac{W_2 - W_1 \text{ (gm}^{-2}\text{ day}^{-1})}{t_2 - t_1}$$

The net assimilation rate (NAR) was calculated by the

$$\text{Net assimilation rate} = \frac{\text{total dry mass}}{\text{leaf area duration}}$$

The concentration of protein was examined.

$$\text{Protein} = \text{Nitrogen} \times 6.25$$

From five randomly selected roots, nodule numbers were counted, and their average was determined (plant$^{-1}$). From five lentil plants, randomly selected root nodules were separated, their weights were measured with an electric balance, and their average was calculated (mg).

## Soil Parameters

The soil nitrate content was assessed by the digestion method (mg kg$^{-1}$). The pre-seeding pH was measured using a soil pH meter [23]. All three trace element contents in lentil seeds were determined by atomic absorption spectrophotometry (AAS). All three trace element contents in lentil plants were determined by atomic absorption spectrophotometry (AAS).

## Cost-Benefit Analysis

A Cost-Benefit analysis was performed using the following equations:

$$\text{Benefit Cost Ratio (BCR)} = \frac{\text{Gross income}}{\text{cost}}$$

$$\text{Gross income} = \frac{\text{Quantity of Main product} \times \text{price of Main product}}{\text{Quantity of Main product} \times \text{price of Main product}}$$

Cost is the total expenses of a gronomic operation in terms of labor, and farm machinery, and all input costs, including seed, pesticides, fertilizers, FYM, etc. All replicated data were inserted for the statistical analyses, and statistical version 8.1 was used to run the experimental design to randomize the complete three factorial design.

# Results and Discussion

A field study was carried out following a randomized complete block design (RCBD) with 3 replications comprising the best eleven combinations of trace elements, along with a control selected after greenhouse experiments to determine nodulation in lentils grown in rainfed areas. The experiment was conducted at the PMAS-Arid Agriculture University research farm, in two consecutive years, i.e., during 2017-18 and 2018-19. Each plot size was maintained at 4.8 m$^2$. The most promising potential yielding lentil variety, NIA Masoor-2005, and the best performing trace element combinations selected after greenhouse experiments were sown with a single row hand drill. Before sowing the crop, soil samples were collected for determination of physico-chemical characteristics (Table 1). After harvesting the lentil crop, the effect of trace element combination application on crop growth, yield contributing parameters, yield, and quality were measured, as well as on soil health. These 12 treatments were used to evaluate the physico-chemical analysis of samples taken from the experimental site. According

Table 1. Physio-chemical analysis of the soil samples from the experimental site.

| Soil Parameters | Means Value (2017-18) | Means Value (2018-19) | Units |
|---|---|---|---|
| Soil pH | 8.1 | 8.1 | |
| EC | 0.21 | 0.22 | $dSm^{-1}$ |
| Bulk density | 1.3 | 1.34 | $gcm^{-3}$ |
| Available P | 6.1 | 6.3 | mg $kg^{-1}$ |
| Extractable K | 76.25 | 75.9 | mg $kg^{-1}$ |
| Nitrate-N | 4.4 | 5.3 | mg $kg^{-1}$ |
| Cobalt | 0.08 | 0.09 | mg $kg^{-1}$ |
| Nickel | 0.15 | 0.17 | mg $kg^{-1}$ |
| Selenium | 0.05 | 0.04 | mg $kg^{-1}$ |
| Soil Organic Carbon | 0.48 | 0.55 | % |
| Clay | 26 | 26 | % |
| Silt | 12 | 12 | % |
| Sand | 62 | 62 | % |
| Textural Class | Sandy-Clay loam | Sandy-Clay loam | |

to treatments, average Soil pH is 8.1, EC is 0.22 dSm-1, Bulk density is 1.3 g cm-3, Available is P 6.1 mg kg-1, Extractable K is 76.25 mg kg-1, Nitrate-N is 4.4 mg kg-1, Cobalt is 0.09 mg kg-1, Nickel is 0.15 mg $kg^{-1}$, Selenium is 0.04 mg $kg^{-1}$, and soil organic carbon is 0.48% while turkey calculated a 42% decrease of SOC [24]. In addition to the physical properties of the soil, the textural class is sandy clay loam and contains 26% clay, 12% silt, and 62% sand [25].

## Effects of Cobalt, Nickel, and Selenium on Morpho-Physiological Characters of Lentil

### *Plant Height*

The height of each plant was measured from the ground level to the apex of the plant at harvest. The data in Fig. 2 show that various combinations of trace elements had a diversified effect on plant height during both years of experimentation. Among all the treatments, the application of Co, Ni, and Se at 600 g $ha^{-1}$ ($T_{12}$) resulted in the maximum plant height (55.2 cm) during both years. The rest of the treatment combinations also performed significantly better than the control ($T_1$) receiving no trace elements. The reason was the favorable environment provided adequate trace element application, which helped the plants grow well. For plant growth and their related bacterial activity and functioning, adequate availability of trace elements such as Co, Ni, and Se for proper plant growth mechanisms is a fundamental process. Their deficiency may limit the development and growth of the plants as well as affect free-living rhizobia in the rhizosphere by damaging their proper functions. Lack of trace element availability is a more serious concern because these elements are unable to recur in alkaline soils due to high pH, resulting in feeble_development of plant cells and hence growth. This could be supported by the findings of [26]. The benefits of trace elements are well documented by various researchers [27-34], and they perform an imperative role in the overall growth process of plants. In different reports, it has been shown that trace elements [34] are essential for the proper process of stem growth and for the expansion of leaf discs that help in stomatal activities during the whole plant growth period. Singh [35] reported that trace elements result in vigorous vegetative growth of legumes, leading to enhanced plant height.

### *Branches Plant$^{-1}$*

On average, the data recorded from experiments over a two-year period showed that the maximum number of branches (10.33 $plant^{-1}$) was recorded from treatment $T_{12}$ (Co, Ni, and Se and 600 g $ha^{-1}$), closely followed by $T_{11}$ (9.50 $plant^{-1}$), which received Co, Ni, and Se and 600, 600, and 300 g $ha^{-1}$, respectively (Fig. 2). Trace elements have numerous beneficial effects on plant growth and development, such as chlorophyll synthesis, consistent stomatal functioning, and encouraging and stimulating growth hormones, which ultimately enhance the branches of crop plants. A significant increase in the number of branches per plant of various crop plants has been well documented by many previous researchers [28, 36]; [30, 34, 37, 38]; [5, 33, 39]. They reported that trace elements play an imperative role in the overall growth process of crop plants [40]. Cobalt (Co) efficiently improves

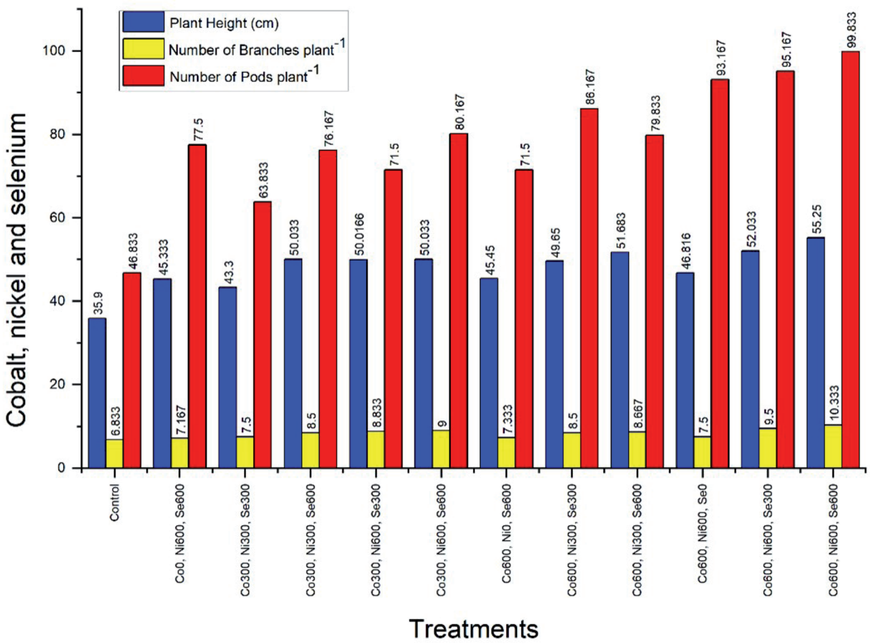


Fig. 2. Plant height, number of branches plant$^{-1}$ , and number of pods plant$^{-1}$ as affected by cobalt, nickel, and selenium.

the growth parameters of tomato plants at all stages of growth, including vegetative, flowering, and fruiting, in two different growing seasons. In addition, selenium (Se) and cobalt (Co) play important roles in physiological processes such as antioxidant activity [41-44].

The minimum number of branches in the control ($T_1$) treatment receiving no trace element application was due to a lack of nutrition in the rhizosphere, which resulted in stunted plant growth and hence reduced branching. Inhibited rooting system architecture causes severe ill effects on plant growth and development due to a lack of nutrition. Deduction in the release of phytohormones leads to enhanced lateral root branching, and root hair formation considerably reduces the uptake of available nutrients, resulting in stunted plant growth [42, 45].

*Number of Pods per Plant$^{-1}$*

The number of pods is the most prominent yield attribute and is most closely correlated with seed yield. It is also the most variable component. To attain the number of pods and number of ears from each pod, they were counted at the harvesting stage compared to unsheathed and counted grains. The results obtained are shown in Fig. 2.

Among all treatments, $T_{12,}$ in which Co, Ni, and Se were applied at 600 g ha$^{-1}$, had the maximum number of pods plant$^{-1}$ (100) during both the 2017-18 and 2018-19 cropping years compared to the control treatment ($T_1$), which received 0 g ha$^{-1}$ Co, Ni, and Se. Comparatively better performance was most likely due to adequate nutrition supplementation, which could be supported by [46], who investigated the effect of cobalt sulfate ($CoSO_4$) on the growth attributes of Faba bean cultivated on sandy loam soil. He reported that the cobalt application showed maximum growth, yield contributing parameters, and yield of Faba bean, i.e., plant height, number of branches plant$^{-1}$, number of nodules, number of pods/plants, number of seed/plants, seed yield freshness and dry weight of the shoot and root. The positive effect of cobalt application might be due to the promotion of many physiological growth processes, such as stem and coleoptile extension, gap of hypostyle hooks, leaf disc expansion, and bud increase. [47] also performed an experiment to study the consequences of cobalt relevance on the growth and yield attributes of lentils. Furthermore, in an experiment, the application of selenium increased the grain yield of lentils, seed Se, and antioxidant levels.

Pods were detached from five randomly selected plants, unsheathed, and sun dried for one week. One thousand seeds were observed at random from the grain lot in every plot and weighed by an electric balance. The treatment ($T_{12}$) supplemented with Co, Ni, and Se at 600 g ha$^{-1}$ showed a maximum 1000-seed weight (35 g) that was effectively higher than the rest of the treatments (Fig. 3).

## Effects of Cobalt, Nickel, and Selenium on Weight, Shoot Dry Weight, and Root Dry Weight

Seed weight is an imperative characteristic of the crop. Even if this character is controlled genetically, the growing condition also exerts influence on its expression. Many studies have shown the positive impact of trace elements on plants. Selenium has a positive effect on the growth and yield of different fruit crops [48]. [49] performed an experiment on sandy loam soil to study the effect of cobalt as cobalt sulfate on various parameters of the Faba bean. They noted that the application of cobalt resulted in maximum levels of growth, yield contributing parameters, and yield of Faba bean plants, i.e., plant height, number of branches, number of nodules, number of pods, number of seeds, seed yield, and fresh and dry weights of shoots and roots of plants. Furthermore, in an experiment, Se increased lentil grain yield, seed Se concentration, and antioxidant levels. The application of Ni is significantly effective on physicochemical and biological parameters with increased soil application [50].

Dry shoot weight was determined at maturity by sampling five plants randomly from each pot. After harvesting the crop on an immediate basis, each sample was weighed to note the fresh weight of the samples. The data in Fig. 3 show that treatment $T_{12,}$ where Co, Ni, and Se were applied at 600 g $ha^{-1}$, yielded a significantly higher dry shoot weight (6.58 g), closely followed by $T_{11}$ (6.28 g), which received Co 600, Ni 600, and Se 300 g $ha^{-1}$, compared to the rest of the treatments. The use of an adequate rate of nutrients is essential for obtaining maximum dry shoot biomass as well as economic yields. Although in this study, each combination did not have a statistically equal effect on yielding maximum dry shoot weight, compared to the control treatment, all combinations of trace elements proved to be significantly superior. [35] concluded that cobalt application along with sulfur produced a higher yield per plant, test weight, protein content, total biomass, and grain yield of lentil crops. They further reported that 4 kg Co $ha^{-1}$ and 20 kg S $ha^{-1}$ should be applied to light textured soils for higher lentil yield production. [51] reported a field study on sandy loam soil and the results of trace nutrients on the growth and yield attributes of beans. Similarly, [41] reported that crop growth parameters were significantly affected by trace element supplementation. [52] reported that cobalt application improved the total biomass of wheat crops. Cobalt sulfate (10 mg $kg^{-1}$) had a greater effect on the dry matter and shoots and roots of wheat. However, higher cobalt levels above 10.0 $mgkg^{-1}$ as cobalt sulfate or cobalt chloride caused a significant reduction in wheat fresh and dry weight production. He determined that the application of trace elements produced higher crop growth and yield.

Root dry weight was estimated at 75 days after sowing (DAS). The data thus obtained are presented graphically in Fig. 3. The data show that treatment $T_{12,}$ where Co, Ni, and Se were applied at 600 g $ha^{-1}$, produced the maximum root dry weight (519.5 mg), closely followed by $T_{11}$ (508.5 mg), which received Co 600, Ni 600, and Se 300 g $ha^{-1,}$ as in the other treatments, and was comparable to its corresponding treatments and control as well. This was probably due to adequate nutrition in the rhizosphere, which provided a favorable environment to flourish the root system as well as plant growth (Figs. 2 and 3). The benefits of trace elements are well documented by various researchers [28, 30, 33, 36, 37, 39, 40, 53, 54], and they play an imperative role in the overall growth process of plants. In different reports, it has been shown that trace elements [34] are essential for the proper process of stem growth and for the expansion of leaf discs that help in stomatal activities during the whole plant growth period. [35] reported that trace elements result in vigorous vegetative growth of legumes, leading to enhanced plant height. [40] reported that cobalt in tomato promotes growth at all stages, such as vegetative, flowering, and fruiting, in the two growing seasons. In addition, selenium (Se) and cobalt (Co) are precursor elements for growth and production, especially in physiological processes commonly associated with antioxidant activity [43, 44, 55, 56].

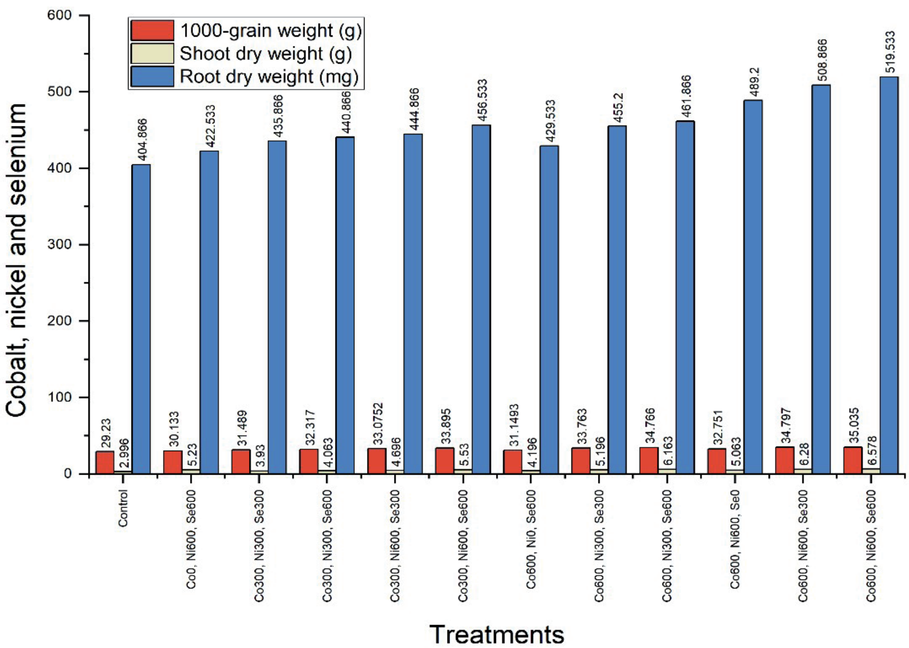


Fig. 3. 1000-grain weight, shoot dry weight, and root dry weight as affected by cobalt, nickel, and selenium.

## Effects of Cobalt, Nickel, and Selenium on Yield and Yield Components

### *Dry Matter Yield*

The results of the consecutive two-year experiments in this study are shown in Fig. 4. The addition of trace elements significantly increased the dry matter yield of lentils compared to the control. The maximum dry matter yield (2146 $kgha^{-1}$) was obtained from treatment $T_{12,}$ where Co, Ni, and Se were applied at 600 $gha^{-1,}$ closely followed by $T_{11}$ (2094 $kgha^{-1}$), receiving Co 600, Ni 600, and Se 300 g $ha^{-1}$ compared to the rest of the treatments, and the minimum was obtained from the control ($T_1$) without any trace element application. This was a nutrient deficiency in the rhizosphere that caused stunted plant growth, resulting in decreased dry matter yield. Overall, a significant increase in dry matter yield was observed due to trace element (Co, Ni, and Se) application. The reason might be adequate nutrition and the involvement of trace elements in plant metabolic processes facilitating photosynthesis, chlorophyll production, pollen functioning, and fertilization mechanisms [57]. A favorable environment in the rhizosphere enhances the activity of a large and diversified microbial species and plant growth.

The root and shoot lengths of soybean were found to be higher at 50 mg $kg^{-1,}$ but they decreased with an increase in cobalt quantity [29]. Plant height increased with increasing levels of cobalt 6 $kgha^{-1}$. [58] also reported that the overall application of Se and Ni increased lentil dry matter yield, nitrogen fixation, antioxidant protection, and seed concentrations. [59] reported that cobalt resulted in a significant increase in biomass production, which may be because Co is responsible for the increase in all minerals. [60] and [61] mentioned the improved root weight for pulses in their study by adding trace elements such as Co, Na, Se, and Ni. They further mentioned that these elements are not critical for all plants but may improve plant growth and yield in certain quantities [42, 61-63]. Our results could also be supported by [64, 65] and [66], who showed the maximum biomass production of lentils through Co, Ni, and Se supplementation compared to other trace elements.

### *Grain Yield*

The data in Fig. 4 shows that the maximum grain yield (1675 $kgha^{-1}$) was harvested from the treatment ($T_{12}$) supplied with Co, Ni, and Se at 600 $gha^{-1,}$ closely followed by $T_{11}$ (1620 $kgha^{-1}$) receiving Co, Ni, and Se at 600, 600, and 300 g $ha^{-1}$, respectively. The rest of the treatments also produced significantly higher grain yields due to various combinations of trace elements compared to the control, where no trace elements were applied. In this case, the reason might be insufficient nutrition in the rhizosphere, which caused an unsatisfactory increase and substandard yield. Our results could be supported by the findings of [64] and [66], in which they showed the maximum grain weight by adding Co, Ni, and Se compared to other trace elements. In many studies, trace elements have been found to be useful for the development of crop plants because they need adequate trace elements for their proper growth. [60] stated that the yield of lentils can be improved by the foliar application of trace elements. Owing to their enhanced enzymatic activity, microelements were efficiently amplified. Translocation and photosynthesis of assimilates into the seed. The highest yield can be attained by foliar spray, which guarantees potential yield production [67]. Similarly, in different studies on Se application to legume crops by [68] and [9]. [10] showed significant improvement in the growth and yields of legume crops, especially lentils. [69] also studied the impact of Co on the yield, nutrients, nodulation, and growth of faba beans. This study shows the improved grain yield, germination of seeds, stand establishment, growth, yield, and quality with the application of trace elements. [58] found that the combined analysis of variance showed that the application of Se fertilizer had a significant effect on grain yield and seed Se concentration. He found an increase in the lentil grain yield of 5% in one year.

### *Total Biomass*

Total biomass is another important trait concerning crops. The results of the successive experiments in this study are shown in Fig. 4. According to the data in the figure, the addition of trace elements significantly amplified the biomass yield compared to the control receiving no trace elements. The maximum biomass yield in year one was (3863 $kgha^{-1}$) harvested from treatment $T_{12}$ in year two supplied with Co, Ni, and Se at 600 g $ha^{-1,}$ followed by $T_{12}$ (3822 kg $ha^{-1}$), receiving Co, Ni, and Se at 600, 600, and 300 g $ha^{-1}$, respectively, and was significantly superior to the rest of the treatments. Reduced biomass yield production was obtained from the control treatment ($T_1$) with no trace element supplementation. In the case of the control treatment, comparatively less biomass yield production was most likely due to the lack of plant nutrition availability in the rhizosphere, which caused stunted plant growth and hence decreased biomass yield production. Our results could be supported by the findings of [64] and in which they showed the maximum grain weight by adding Co, Ni, and Se compared to other trace elements. In many studies, trace elements have been found to be beneficial_for the development of crop plants because they need adequate trace elements for their proper growth. [34, 60] stated that the yield of lentils can be improved by the foliar application of trace elements. Owing to their enhanced enzymatic activity, microelements significantly improve plant growth and crop yields as well. [70] reported that potential crop yields could be achieved_through the foliar application of trace elements. Similarly, different studies on Se application to legume crops by [8] and [71] showed significant improvement in the growth and yields of legume crops, especially lentils. [46] also studied the encouraging impact of Co on the growth and yield of faba beans. In another study, [72] also reported an increased biomass by adding cobalt. Various reports show that Se and Co added at minimum concentrations exert beneficial effects on plant growth. Selenium may act as a quasiessential micronutrient by varying different physiological and biochemical traits. [9] also documented similar conclusions. Among the essential micronutrients for soybeans, supplementation with Co, Ni, and Se significantly improved the biological yield. [73] also concluded that Ni promotes plant growth and biomass, although it depends on soil clay particles. However, other studies have reported improvements in the yield and biomass of crops after Se application [74].

## Effects of Cobalt, Nickel, and Selenium on Harvest Index, Leaf Area, and Leaf Area Index

### *Harvest Index*

The harvest index is a measure of the efficiency of plants in producing seeds. During two consecutive study years, 2017-18 and 2018-19, according to the data in the figure, the addition of trace elements significantly amplified the harvest index (HI) compared to the control receiving no trace elements (Fig. 5). The maximum HI was recorded from the $T_7$ treatment, among all other treatments. The minimum HI was determined for the control treatment ($T_{12}$). This was certainly_due to insufficient nutrition in the rhizosphere that caused stunted plant growth (Fig. 2) and hence grain yield (Fig. 4), resulting in a decreased HI of the lentil crop during both years of study. Overall, a slight increase in the HI of the lentil crop was observed due to trace element (Co, Ni, and Se) application. The reason might be adequate nutrition and the involvement of trace elements in plant metabolic processes, facilitating photosynthesis activity, chlorophyll production processes, and hence pollen functioning and fertilization mechanisms. A similar discussion was also made by [57]. A favorable environment in the rhizosphere enhances the activity of microbes to promote plant growth and crop yield.

The benefits of trace elements are well documented by various researchers [28, 33, 36, 37, 39, 53, 64], who reported that trace elements perform an imperative role in the overall plant growth process and ultimately crop yields. In different reports [5, 34, 54], it has been shown that trace elements are essential for the proper functioning processes of plant growth and for the expansion of leaf discs that help in stomatal activities during the whole plant growth period. [35] reported that trace elements result in vigorous vegetative growth of legumes, leading to enhanced yields. In addition, Se and Co are considered useful elements for growth and yield [43, 75].

### *Leaf Area*

Data from two years of experimentation were recorded and are graphically presented in Fig. 5. It is clear from the data that various combinations of trace elements significantly improved the LA compared to the control treatment ($T_1$) receiving no trace elements. The maximum LA (366 $cm^{-2}$) was detected in treatment $T_{12}$ supplied with Co, Ni, and Se at 600 g $ha^{-1,}$ followed by $T_{11}$ (349 $cm^{-2}$), which received Co, Ni, and Se at 600, 600, and 300 g $ha^{-1}$, respectively, during both

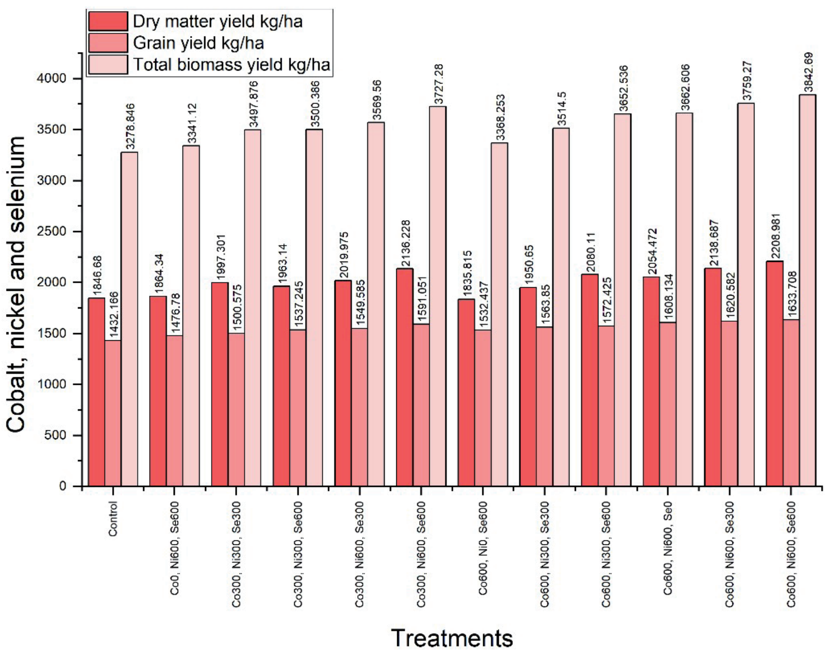


Fig. 4. Dry Matter Yield, Grain Yield, and Total Biomass as affected by cobalt, nickel, and selenium.

years of the study and was significantly superior to the other treatments. The minimum LA was obtained from the control treatment ($T_1$) with no trace element supplementation, which was most likely due to the lack of plant nutrition availability in the rhizosphere, which caused stunted plant growth and hence reduced LA. A plant with optimum LA may produce a higher biological yield. [29] found that a higher dose of Se caused a significant reduction in the LA of *Zea mays*. Resembling interpretations have also been reported by [76]. [50, 64] and [34] showed the maximum plant growth attributes by adding Co, Ni, and Se compared to other trace elements. In many studies, trace elements have been found to be beneficial for the development of crop plants because they need adequate trace elements for their proper growth. [60] stated that lentil yield can be improved by improving growth and yield, contributing parameters through foliar application of trace elements. Owing to their enhanced enzymatic activity, microelements significantly improve plant growth and crop yields as well.

### *Leaf Area Index*

The leaves of the plant are the most important photosynthetic shoots. Plants were harvested, and the leaf area index was calculated. Data from two years of experimentation were recorded and are graphically presented in Fig. 5. It is clear from the data that various combinations of trace elements significantly improved the LAI compared to the control treatment ($T_1$) receiving no trace elements. The maximum LAI (2.25) was detected in treatment $T_{12}$ supplied with Co, Ni, and Se at 600 g ha$^{-1,}$ followed by $T_{11}$ (2.24), which received Co, Ni, and Se at 600, 600, and 300 g ha$^{-1}$, respectively, during both years of the study and was significantly superior to the other treatments. The minimum LAI was obtained from the control treatment ($T_1$) with no trace element supplementation, which was most likely due to the lack of plant nutrition availability in the rhizosphere, which caused stunted plant growth and hence reduced LAI. A plant with optimum LAI may produce a higher biological yield. [29] conducted a pot experiment on *Zea mays* and found that a higher dose of Se caused a significant reduction in LAI. [70] reported that potential crop yields could be achieved_through the foliar application of trace elements. Similarly, different studies on Se application to legume crops showed significant improvement in the growth and yields of legume crops [9, 71]. [46] also studied the encouraging impact of Co on the growth and yield of faba beans. In another study, [77] also reported increased biomass by adding Co. Various reports show that Co, Ni, and Se applications exert beneficial effects on plant growth. Selenium may act as a quasiessential micronutrient by varying different physiological and biochemical traits. [78] also concluded that nickel promotes plant biomass production.

## Effect of Cobalt, Nickel, and Selenium on the Crop Growth Rate, Net Assimilation Rate, and Grain Protein Content

### *Crop Growth Rate (CGR)*

The crop growth rate (CGR) was recorded during the whole crop growth period, starting from the time of germination until the maturity of the lentil crop. The data in Fig. 6 clearly indicated that the highest growth rate (5.6 gm$^{-2}$ day$^{-1}$) was observed with the

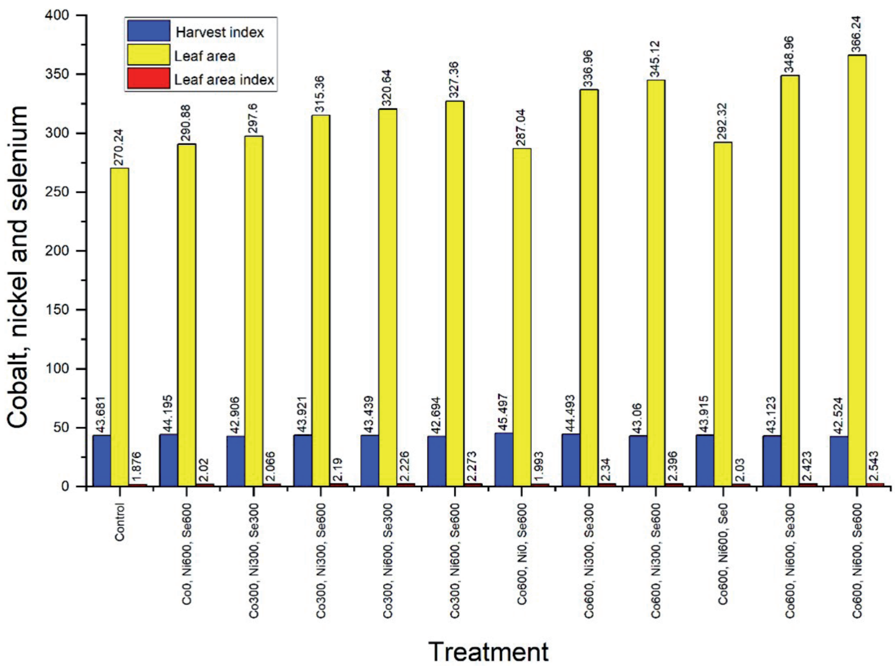

Fig. 5. Harvest index, leaf area, and leaf area index as affected by cobalt, nickel, and selenium.

application of Co, Ni, and Se at 600, 600, and 300 g $ha^{-1}$, respectively, followed by $T_{12}$ (5.4 $gm^{-2}$ $day^{-1}$), which received Co, Ni, and Se at 600 g $ha^{-1}$, respectively, during both years of study and was significantly superior to the rest of the treatments. However, all the combinations of Co, Ni, and Se applications significantly improved CGR compared to the control treatment ($T_1$) which received no trace elements but was substantially less than that of $T_{12}$ and $T_{11}$. Our results could also be supported by the findings of [64] and [34], in which they showed the maximum plant growth attributes by adding Co, Ni, and Se compared to other trace elements. In many studies, trace elements have been found to be beneficial for the development of crop plants because they need adequate trace elements for their proper growth.[60] stated that lentil yield can be improved by improving growth and yield contributing parameters through foliar application of trace elements. Owing to their enhanced enzymatic activity, microelements significantly improve plant growth and crop yields as well. [70] reported that potential crop yields could be achieved_through the foliar application of trace elements. Similarly, different studies on Se application to legume crops have shown significant improvements in the growth and yields of legume crops [9, 68, 71, 76]. They also studied the encouraging impact of Co on the growth and yield of faba bean. In another study, [72] also reported increased biomass by adding Co. Various reports show that Co, Ni, and Se applications exert beneficial effects on plant growth. Selenium may act as a quasiessential micronutrient. Initially, Ni was recognized as an essential nutrient for plant growth and the completion of the plant life cycle. [79] found an increase in lentil CGR by adding Se to Se-deficient soils in Bangladesh. Our results could also be supported by the findings of [80].

*Net Assimilation Rate*

The net assimilation rate was recorded during the growth periods 2017-18 and 2018-19 of the lentil crop. The data in Fig. 6 clearly indicate that the maximum NAR (6.07 $gm^{-2}$ $day^{-1}$) was observed with the application of Co, Ni, and Se at 600 g $ha^{-1,}$ followed by $T_{11}$ (5.57 $gm^{-2}$ $day^{-1}$) receiving Co, Ni, and Se at 600, 600, and 300 g $ha^{-1}$, respectively, during both years of study and was significantly superior to the rest of the treatments. However, all the combinations of Co, Ni, and Se applications significantly improved NAR compared to the control treatment ($T_1$) which received no trace elements but was substantially less than that of $T_{12}$ and $T_{11}$. [64] and [66] investigated maximum plant growth attributes by adding Co, Ni, and Se compared to other trace elements. In many studies, trace elements have been found to be beneficial for the development of crop plants because they need adequate trace elements for their proper growth. [60] stated that lentil yield can be improved by improving growth and yield contributing parameters through foliar application of trace elements. Owing to their enhanced enzymatic activity, microelements significantly improve plant growth and crop yields as well. [70] reported that potential crop yields could be achieved through the foliar application of trace elements. Similarly, different studies on Se application to legume crops showed significant improvement in the growth and yields of legume crops [9, 71, 81]. [46] also studied the encouraging impact of Co on the growth and yield of faba beans. In various reports [82-84], it has been shown that Co, Ni, and Se applications exert beneficial effects on plant growth. Selenium may act as a pseudoessential micronutrient throughout the crop growth period. Initially, Ni was recognized as an essential nutrient for plant growth and the completion of the plant life cycle.

*Grain Protein Content*

Grain protein content (%) was determined from grain samples collected after the crop harvest. The results presented in Fig. 6 clearly indicate that the maximum percentage of grain protein (26.2%) was recorded from treatments ($T_{12}$) supplemented with Co, Ni, and Se at 600 g $ha^{-1}$ and ($T_{11}$) receiving Co, Ni, and Se at 600, 600, and 300 g $ha^{-1}$, respectively, which were statistically at par during both years of study and were significantly superior to the rest of the treatments. The lowest protein content, in the case of the control treatment having no trace elements, was due to nutrient deficiency in the rhizosphere, resulting in a comparatively decreased percentage of grain protein content. Protein content is used as a pointer to environmental conditions for learning about varietal and environmental differences in the proximate symphony, minerals, amino acids, and anti-nutrients of lentils [85]. [76] reported that the protein content in grain was significantly improved with the addition of Co (8 kg $ha^{-1}$) and sulfur (40 kg $ha^{-1}$). Another study performed by [76] demonstrated that N- and Coefficient soils have low protein and ultimately rescue yield. Similarly, [66] reported maximum plant growth and yield with better quality by adding Co, Ni, and Se compared to other trace elements. In many other studies, trace elements have been found to be beneficial_for the development of crop plants and improved quality owing to adequate trace element application. [60] stated that the yield and quality of lentils could be improved by improving growth and yield contributing parameters through the foliar application of trace elements. They further reported that trace elements enhance the enzymatic activity of crop plants owing to their proper functioning as a result of satisfactory availability, and microelements significantly improve plant growth and crop yields as well. [70] reported that potential crop yields with better quality could be achieved_through the foliar application of trace elements. [34] reported that trace elements such as cobalt, nickel, copper, selenium, molybdenum, cadmium, and barium have a significant influence on the seed protein content of lentils.

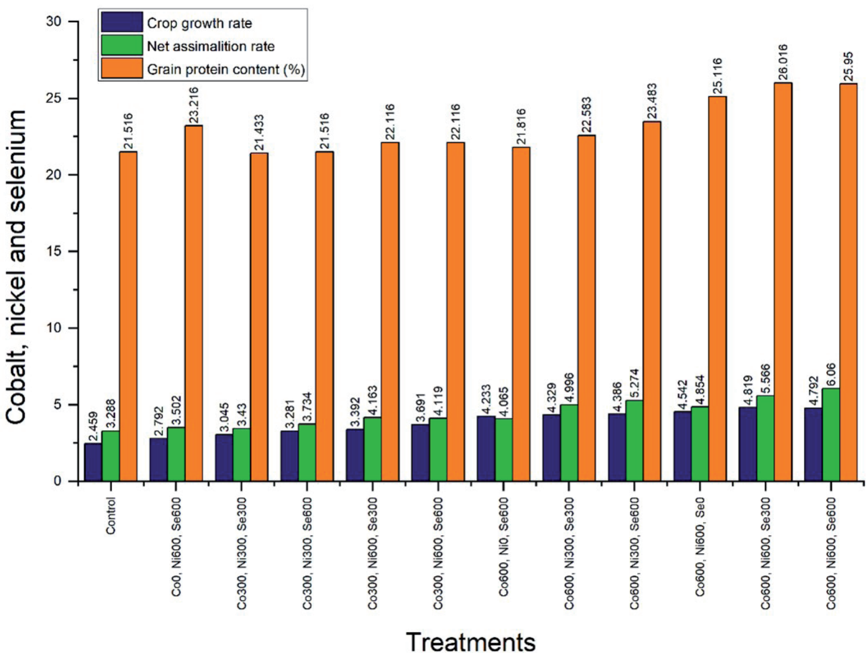


Fig. 6. Crop growth rate, net assimilation rate, and grain protein content as affected by cobalt, nickel, and selenium.

## Effect of Cobalt, Nickel, and Selenium on the Nodule Formation of Lentil

### *Number of Nodules Plant$^{-1}$*

The number of nodules plants$^{-1}$ randomly selected from five roots as affected by Co, Ni, and Se applications was recorded as shown in Fig. 7. It is clear from the data that the maximum number of nodules (36.33) was recorded from treatments ($T_{12}$) supplemented with Co, Ni, and Se at 600 g ha$^{-1}$ and ($T_{11}$) (35) receiving Co, Ni, and Se at 600, 600, and 300 g ha$^{-1}$, respectively, which were statistically at par during both years of study and were significantly superior to the rest of the treatments. The lowest number of nodules, in the case of the control treatment having no trace elements, was definitely due to the absence of trace elements in the rhizosphere, resulting in comparatively declined nodule formation. [61] reported that Co is a necessary component of cobalamin, which is required for the activities of several enzymes and coenzymes and is responsible for the formation of leghemoglobin, which enhances nodulation. Root nodulation was also affected by Ni and Co concentrations in the root zone, showing a corresponding increase in the total number of nodules with adequate supplementation of both metals.

Root nodule bacteria need access to sufficient concentrations of mineral nutrients. The developmental process of some legume symbioses specifically requires micronutrients. It is stated in his paper that rhizobium symbiosis requires micronutrients, including boron, cobalt, copper, molybdenum, manganese, and zinc (among others), sometimes at higher rates than the free-living plant or bacteria alone. Co is an essential nutrient in rhizobia and is a requirement for nitrogen fixation [86]. A 67% increase in the number and weight of nodules was recorded over the control, owing to the sole application of Co. It seems that trace elements have a significant contribution to nodule formation, their development, and functions, ultimately providing vigorous growth for host plants. Our results could be supported by the findings of [87] that cobalt sulfate significantly raised the physiological attributes and its efficacy due to higher nitrogenase, especially 100 and 75% of N fertilizers in groundnut crops. Moreover, significantly ($p \leq 0.05$) higher numbers and dry weights of nodules were observed when Co was applied at a rate of 0.21 kg ha$^{-1}$ [40, 76]. Previous research by [76] reported similar findings. Another team of researchers, Nasser et al. (2008), also documented that micronutrients are necessary and important for the synthesis and activity of enzymes such as the nitrogen adaptation enzyme nitrate reductase and the nitrogen-fixing enzyme nitrogenase. Indeed, there are numerous reports that legume responses to trace element supplementation have a direct positive influence on chlorophyll content, shoot growth, number of nodules, seed quality, and nutrient use efficiency [43, 66, 76, 86, 88].

### *Weight of Nodules*

The weights of nodules plants$^{-1}$ randomly selected from five roots as affected by Co, Ni, and Se applications were recorded as shown in Fig. 7. It is clear from the data that the maximum weight of nodules (31.96 mg) was recorded from treatments ($T_{12}$) supplemented with Co, Ni, and Se at 600 gha$^{-1}$ and ($T_{11}$) (31.09) receiving Co, Ni, and Se at 600, 600, and 300 gha$^{-1}$, respectively, which were statistically at par during both years of study and were significantly superior to the rest of the treatments.

The lowest number of nodules; in the case of the control treatment having no trace elements, was definitely due to the absence of trace elements in the rhizosphere, resulting in comparatively declined nodule formation. [61] reported that Co is a component of cobalamin, which is used for the activities of other enzymes and is responsible for the formation of leghemoglobin to increase nodulation. Root nodulation was also affected by Ni and Co concentrations in the root zone, showing a corresponding increase in the total number of nodules with adequate supplementation of both metals.

*Soil Nitrate Content*

The trace element supplemented treatments significantly influenced soil nitrate after harvest of the lentil crop (Fig. 7), with the maximum in treatments ($T_{12}$) supplemented with Co, Ni, and Se at 600 g $ha^{-1}$ and ($T_{11}$) receiving Co, Ni, and Se at 600, 600, and 300 $gha^{-1}$, respectively, which were statistically significantly superior to other treatments. The control treatment ($T_1$) showed a minimum level of total nitrate nitrogen, which might be due to its utilization by the lentil crop for its growth. It is generally believed that legume crops as well as nodule formation processes utilize native nitrate nitrogen during their growth, causing a significant depletion in soil. The lentil crop sown with trace element application sparingly impressed the level of soil nitrate nitrogen determined after harvest. This was presumably because trace element supplementation provided the nutrient requirements for adequate crop growth and maintained higher levels. On the other hand, legume crops respond better to maintaining the fertility level of the soil and show comparatively less diminution in the soil nitrate nitrogen level. Legume crops can improve soil fertility, especially through nitrogen fixation in nitrogen-deficient soils. Detailed discussion regarding soil fertility restoration has also been documented by various early researchers [89].

After the harvest of the lentil crop, the nitrate nitrogen status improved and was relatively higher compared to the control receiving no trace elements. It is clear from the results in Fig. 7 that under the control treatment, without any trace element application, the nitrate level was much lower than that in the treatments supplied with trace elements. Trace elements such as Co, Ni, and Se are good supporters of soil microflora, which play a vital role in preserving soil fertility. The microorganism concerned with scavenging these trace elements significantly improved the growth and yield of lentils by escalating the effectiveness of biological nitrogen fixation in addition to easing access to macro- and micronutrients such as N, P, K, Fe, Zn, Co, Ni, and Se through the fabrication of plant growth-promoting substances in the rhizosphere. [40, 84] and [90] documented similar findings. They reported that supplementation with Co at 0.10 ppm is ideal for restoring soil fertility. Cobalt is occupied by the fixation of atmospheric N by Rhizobium bacteria and promotes the functions of a variety of beneficial soil bacteria [28, 76].

## Effect of Cobalt, Nickel, and Selenium on the Seed Cobalt, Nickle, and Selenium Content

Trace element (Co, Ni, and Se) concentrations in lentil seeds determined after the crop harvest are presented in Fig. 8. The data indicates that the maximum

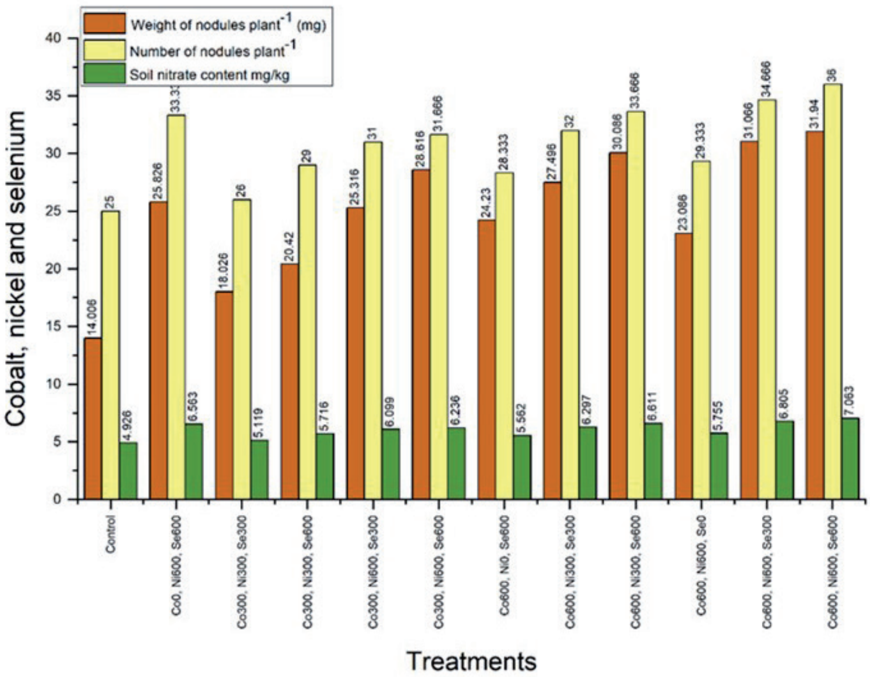


Fig. 7. Weight of nodules, number of nodules plant-1, and soil nitrate content as affected by cobalt, nickel, and selenium.

concentrations of Co, Ni, and Se were determined from the seeds harvested from treatments where Co, Ni, and Se were applied at 600 g ha$^{-1}$ compared to their lower rates (300 g ha$^{-1}$). The minimum concentrations of these elements (Co, Ni, and Se) were determined from the control treatment ($T_1$), where no trace elements were applied. This could most likely be the reason that, with adequate trace element supplementation, the lentil crop produced higher grain yield and total biomass when treated with a higher rate of trace elements (Fig. 4). Earlier research workers reported similar findings. [91] added that cobalt recorded the maximum nodulation in groundnut roots. [76] noted that cobalt at 8 ppm increased total nodule number and dry weight, the number and weight of effective nodules, and root dry weight in pea (*pisumsativum L*) plants. [92] reported that cobalt increased the number and weight of cowpea nodules and the leghaemglobin content of root nodules. [93] found that 50 mg per kg cobalt in soil improved growth parameters in groundnut and soybean. [94] showed that cobalt acts as an essential element for certain microorganisms, and fixing atmospheric N for nodule formation improves the efficiency of N fixation in legume crops.

Naturally, plants uptake more nutrients available in the root zone for their proper growth. Nickel is naturally present in soil and water, usually in trace amounts. Several plants nutritionally require Ni for various metabolic activities. Ni (<5 $\mu$g L$^{-1}$) may stimulate growth in higher plants [95]. Excess concentrations of Ni may cause numerous adverse effects on flora. The physiological role of Ni in higher plants has been observed [96-99]. Most plants were adversely affected by concentration levels above 50 µgNig$^{-1}$ dry weight in tissues. These effects are manifested at morphological, physiological, and biochemical levels, and they may result either because of the tendency of Ni to compete with other cations such as $Ca^{2+}$, $Fe^{2+}$, and $Zn^{2+}$ and thus cause their artificial deficiencies. More nickel than its range in the soil and plant tissue causes a deficiency of Zn or Fe, which causes symptoms of chlorosis [100].

[101] found that legumes have an important role in overcoming nutrient deficiency and malnutrition in developing countries' food security. A literature review describes genotype effects on seed mineral concentration in lentils. This experiment was also performed to increase the seed mineral contents, and according to the results, the full dosage of Ni provided to the soil gave a high seed content with maximum uptake. There are many reports that support our findings that high concentrations in the root zone favor significant storage of nutrient_elements in the seed content. [76] reported that the uptake of trace elements (Co, Ni, and Se) in lentil grain and straw increased considerably with increasing levels of trace element application. In another study, a significant increase in the yield of legumes with the application of cobalt was reported by [92].

## Effect of Cobalt, Nickel, and Selenium on the Straw Cobalt, Nickle, and Selenium Content of Lentil

Trace element (Co, Ni, and Se) concentrations in lentil tissue determined after the crop harvest had similar trends as those in lentil straw (Fig. 9). The data indicate that maximum concentrations of Co, Ni, and Se were observed in lentil tissues harvested from treatments where Co, Ni, and Se were applied at higher rates (600 gha$^{-1}$). It has been discussed that the reason could most likely be adequate trace element supplementation leading to higher grain yield and total biomass

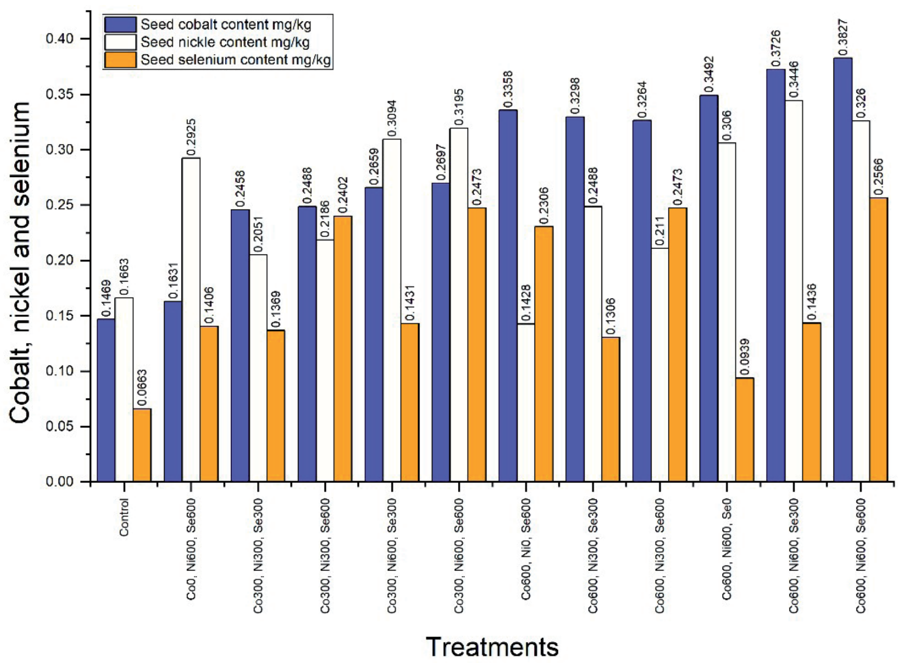


Fig. 8. Seed Cobalt, Nickle, and Selinium Content by cobalt, nickel, and selenium.

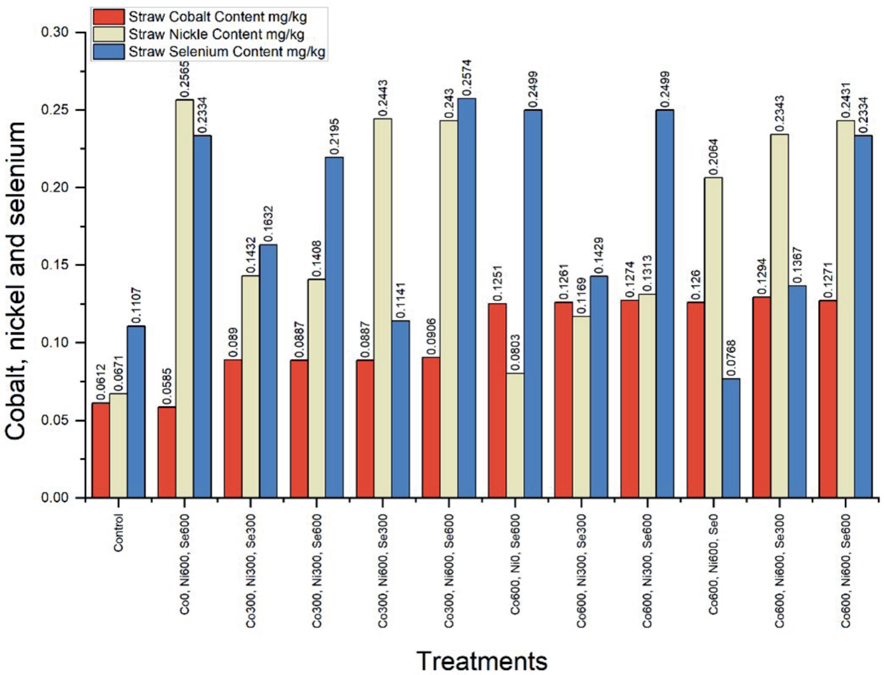


Fig. 9. Straw Cobalt, Nickle, and Selenium Content by cobalt, nickel, and selenium.

production due to more uptake of these elements under elevated rates of trace elements (Fig. 4). Earlier research [76, 92, 93, 102-106] documented similar findings. They reported that trace element (Co, Ni, and Se) application improved nodulation in roots, total biomass, and seed yield owing to adequate uptake of trace elements in grain as well as in plant tissues of different legume crops (lentil. cowpea; *Pisum sativum* L., groundnuts, soybean). They further reported that increases in growth parameters such as plant height, root length, total leaf area, shoot and root dry weights, and pod yield of legumes were presumably due to sufficient supply and consumption of trace elements in plant tissues. Trace elements are essential for certain microorganisms, especially those fixing atmospheric nitrogen for nodule formation, and their deficiency seems to depress the efficiency of N fixation in legume crops. There are many reports that support our findings that high concentrations in the root zone favor significant storage of nutrient elements in the seed content. [76] reported that the uptake of trace elements (Co, Ni, and Se) in lentil grain and straw increased considerably with increasing levels of trace element application. In another study, a significant increase in the yield of legumes with the application of cobalt was reported by [92].

## The Benefit Cost Ratio of Cobalt, Nickel, and Selenium

The cost benefit ratio (BCR) was computed. These results are encouraging and worth recommending. The data in Table 2 indicate that the maximum cost benefit ratio (BCR) for lentil crops (1.77) grown under treatment $T_{10}$ (Co, Ni, and Se at 600, 600, and 0 g ha$^{-1}$) was closely followed by 1.75 in $T_{11}$ (600, 600, and 300 g ha$^{-1}$) and 1.74 in $T_{12}$ (600 g ha$^{-1}$ each). Among all trace element supplemented treatments, the lowest BCR of 1.58 for lentils was calculated from treatment $T_2$ receiving Co, Ni, and Se at 0, 600 and 600 g ha$^{-1}$. Generally, the trace element application in all combinations performed the best except $T_2$ (Co, Ni, and Se at 0, 600, and 600 g ha$^{-1}$), which might be due to the absence of Co and the higher rates (600 g ha$^{-1}$) of Ni and Se, which might have caused a declined BCR. Much higher net income (Rs = 49102/-) in the case of treatments $T_{10}$ (Co, Ni, and Se at 600, 600, and 0 g ha$^{-1}$), closely followed by $T_{11}$ (Rs = 48754/-) and $T_{12}$ (Rs = 48475/-), might be due to adequate nutrition supplementation resulting in comparatively higher net income. Consequently, nutrient utilization efficiency positively affected the healthy growth and yields of lentil crops and, thus, net income. This could be supported by the findings of similar points of view regarding a better correlation between trace element nutrition and plant growth and economic yields. In another study by [46], a similar trend in economic analysis results of faba bean cultivars and nutrient application rates was observed. The results of [107] showed a better correlation between nutrient application and the growth and yield of pea (*Pisum sativum* L.). They further reported that the application of inorganic fertilizers integrated with farmyard manure enhanced nutrient availability and improved the economical production of mung beans with better net income. These findings could also be supported by the results of [108]. Based on all economic analyses of the study over two years (2017-18 and 2018-19), trace element supplementation could be recommended to farmers to obtain maximum net return.

Table 2. Benefit cost ratio of cobalt, nickel, and selenium.

| Treatments ($g\ ha^{-1}$) | Grain yield ($kg\ ha^{-1}$) | Gross income (Rs.) | Expenses (Rs.) | Net income (Rs) | Additional exp. (Rs.) | Value of increased yield (Rs.) | BCR |
|---|---|---|---|---|---|---|---|
| Control | 1432 | 100252 | 62125 | 38127 | Nil | Nil | 1.61 |
| $Co_0Ni_{600}Se_{600}$ | 1477 | 103375 | 65432 | 37943 | 3307 | 3123 | 1.58 |
| $Co_{300}Ni_{300}Se_{300}$ | 1501 | 105040 | 64015 | 41025 | 1890 | 4789 | 1.64 |
| $Co_{300}Ni_{300}Se_{600}$ | 1537 | 107607 | 65234 | 42373 | 1890 | 7356 | 1.65 |
| $Co_{300}Ni_{600}Se_{300}$ | 1550 | 108471 | 64450 | 44021 | 2325 | 8219 | 1.68 |
| $Co_{300}Ni_{600}Se_{600}$ | 1591 | 111374 | 65669 | 45705 | 3544 | 11122 | 1.70 |
| $Co_{600}Ni_0Se_{600}$ | 1532 | 107271 | 65036 | 42234 | 2911 | 7019 | 1.65 |
| $Co_{600}Ni_{300}Se_{300}$ | 1564 | 109470 | 65471 | 43999 | 3346 | 9218 | 1.67 |
| $Co_{600}Ni_{300}Se_{600}$ | 1572 | 110070 | 64252 | 45818 | 2127 | 9818 | 1.71 |
| $Co_{600}Ni_{600}Se_0$ | 1608 | 112569 | 63467 | 49102 | 1342 | 12318 | 1.77 |
| $Co_{600}Ni_{600}Se_{300}$ | 1621 | 113441 | 64686 | 48754 | 2561 | 13189 | 1.75 |
| $Co_{600}Ni_{600}Se_{600}$ | 1634 | 114380 | 65905 | 48475 | 3780 | 14128 | 1.74 |

## Conclusion

A two-year consecutive field study spanning the agricultural seasons of 2017-18 and 2018-19 was carried out to explore optimal trace element (Co, Ni, and Se) application methods, either in single or in combination, for enhancing lentil (MIA Masoor-2005 variety) cultivation. The lentils were sown using a single row hand drill, and the trace elements were applied either individually or combined through fertigation post lentil sowing. Superior crop growth, enhanced yield, and improved quality were obtained with the application of Co, Ni, and Se at 600 $gha^{-1}$ followed closely by treatments involving Co, Ni, and Se at 600, 600, and 300 $g/ha^{-1}$, respectively. The maximum grain yield (1638 $kgha^{-1}$) was harvested from the treatment where Co, Ni, and Se were applied at 600 $gha^{-1,}$ which was 15% higher than that of the control receiving no trace elements.

The most effective treatment applied full doses of nickel and cobalt, each applied at 600 g/ha-1. The application of Co and Ni @ 600 $g/ha^{-1}$ emerged as the most economically viable option, showing comparable results regarding growth, nodulation, and yield contributing parameters when compared to the sole application of Co, Ni, and Se at 600 $gha^{-1}$.

The use of trace elements (Co, Ni, and Se) either individually or in combination, proves to be an economical strategy with the potential to significantly increase both the yield and nodulation of lentils [109]. Demonstrated the positive response to higher doses in lentils; also, according to the researcher, these findings underscore the importance of monitoring and managing plant species used to mitigate potential health risks associated with Ni [110]. Further research is needed to determine the optimal doses of these trace elements. Additionally, cost-effective methods, such as foliar application, may be formulated for practical implementation.

## Data Availability Statement

The authors declare that data supporting the findings of this study are available on request from the corresponding author.

## Conflict of Interests

The authors declare that they have no conflict of interest.


## Funding

The authors would like to extend their sincere appreciation to the Researchers Supporting Project Number (RSP2024R134), King Saud University, Riyadh, Saudi Arabia.

## Acknowledgment

The authors would like to extend their sincere appreciation to the Researchers Supporting Project Number (RSP2024R134), King Saud University, Riyadh, Saudi Arabia.

## References


1. TAALAB A., BADR M. Phosphorus availability from compacted rock phosphate with nitrogen to sorghum inoculated with phospho-bacterium. Journal of Applied Sciences Research, **3** (3), 195, **2007**.
2. AKSOY T., CETIN M., CABUK S.N., SENYEL KURKCUOGLU M.A., BILGE OZTURK G., CABUK A. Impacts of wind turbines on vegetation and soil cover: a case study of Urla, Cesme, and Karaburun Peninsulas, Turkey. Clean Technologies and Environmental Policy, **25** (1), 51, **2023**.
3. COOK R.J. Advances in plant health management in the twentieth century. Annual review of phytopathology, **38** (1), 95, **2000**.
4. MASSOURA S.T., ECHEVARRIA G., BECQUER T., GHANBAJA J., LECLERC-CESSAC E., MOREL J.-L. Control of nickel availability by nickel bearing minerals in natural and anthropogenic soils. Geoderma, **136** (1-2), 28, **2006**.
5. CHATTERJEE R., BANDYOPADHYAY S. Effect of boron, molybdenum and biofertilizers on growth and yield of cowpea (*Vigna unguiculata* L. Walp.) in acid soil of eastern Himalayan region. Journal of the Saudi Society of Agricultural Sciences, **16** (4), 332, **2017**.
6. KHUNTIA D., PANDA N., MANDAL M., SWAIN P., SAHU S., PATTANAYAK S. Symbiotic effectiveness of acid tolerant nodulating rhizobia on growth, yield and nutrient uptake of pigeon pea (*Cajanus cajan* l.) in acidic Alfisols. International Journal of Bio-resource and Stress Management, **13** (4), 403, **2022**.
7. FENG T., CHEN S., GAO D., LIU G., BAI H., LI A., PENG L., REN Z. Selenium improves photosynthesis and protects photosystem II in pear (Pyrus bretschneideri), grape (*Vitis vinifera*), and peach (*Prunus persica*). Photosynthetica, **53**, 609, **2015**.
8. ZHANG X., LAUBIE B., HOUZELOT V., PLASARI E., ECHEVARRIA G., SIMONNOT M.-O. Increasing purity of ammonium nickel sulfate hexahydrate and production sustainability in a nickel phytomining process. Chemical engineering research and design, **106**, 26, **2016**.
9. WANG J., WANG Z., MAO H., ZHAO H., HUANG D. Increasing Se concentration in maize grain with soil-or foliar-applied selenite on the Loess Plateau in China. Field Crops Research, **150**, 83, **2013**.
10. THAVARAJAH D., THAVARAJAH P., KUMAR S., COMBS G.F. Will selenium increase lentil (Lens culinaris Medik) yield and seed quality? Frontiers in Plant Science, **6**, 140475, **2015**.
11. KARAN D., SINGH S. Effect of zinc and boron application on yield of lentil and nutrient balance in the soil under Indo-Gangetic Plain zones. Journal of AgriSearch, **1**, (4), **2014**.
12. CHAKRABORTY A. Growth and yield of lentil (*Lens culinaris* L.) as affected by Boron and Molybdenum application in lateritic soil. Journal of Crop and Weed, **5** (1), 88, **2009**.
13. BASU T. Effect of Cobalt, Rhizobium and Phosphobacterium Inoculations on Growth, Yield, Quality and Nutrient Uptake of Summer Groundnut (Arachis hypogaea). American Journal of Experimental Agriculture, **1** (1), 21, **2010**.
14. ZEIDAN M., HOZAYN M., ABD EL-SALAM M. Yield and quality of lentil as affected by micronutrient deficiencies in sandy soils. Journal of Applied Scientific Research, **2** (12), 1342, **2006**.
15. MONTENEGRO J.B.V., FIDALGO J.A.B., GABELLA V.M. Response of chickpea (*Cicer arietinum* L.) yield to zinc, boron and molybdenum application under pot conditions. Spanish Journal of Agricultural Research, (3), 797, **2010**.
16. AISHA A.E.S.A. Determination of boron for indoor architecture plants used in indoor architectural designs. Scientific Research Communications, **3** (2), **2023**.
17. QUDDUS M., MIAN M., NASER H., HOSSAIN M., SULTANA S. Maximizing yields, nutrient uptake and balance for Mustard-Mungbean-T. Aman rice cropping systems through nutrient management practices in calcareous soils. Journal of Agriculture Science, **9**, 210, **2017**.
18. CETIN M., JAWED A.A. The chancing of Mg concentrations in some plants grown in pakistan depends on plant species and the growing environment. Kastamonu University Journal of Engineering and Sciences, **7** (2), 167, **2021**.
19. CETIN M., ISIK PEKKAN O., BILGE OZTURK G., SENYEL KURKCUOGLU M.A., KUCUKPEHLIVAN T., CABUK A. Examination of the change in the vegetation around the Kirka Boron mine site by using remote sensing techniques. Water, Air, & Soil Pollution, **233** (7), 254, **2022**.
20. CETIN M., ALJAMA A.M.O., ALRABITI O.B.M., ADIGUZEL F., SEVIK H., ZEREN CETIN I. Determination and mapping of regional change of Pb and Cr pollution in Ankara city center. Water, Air, & Soil Pollution, **233** (5), 163, **2022**.
21. CETIN M., ALJAMA A.M.O., ALRABITI O.B.M., ADIGUZEL F., SEVIK H., ZEREN CETIN I. Using topsoil analysis to determine and map changes in Ni Co pollution. Water, Air, & Soil Pollution, **233** (8), 293, **2022**.
22. TIARKS A., RANGER J. Soil properties in tropical plantation forests: evaluation and effects of site management: a summary. Site management and productivity in tropical plantation forests. Edited by EKS Nambiar. Centre for International Forestry Research, Jayakarta, Indonesia, 191, **2008**.
23. QUIGLEY M.N. Testing soils for lime requirement. CRC Press, **2020**.
24. CETIN M., ISIK PEKKAN O., BILGE OZTURK G., CABUK S.N., SENYEL KURKCUOGLU M. A., CABUK A. Determination of the Impacts of Mining Activities on Land Cover and Soil Organic Carbon: Altintepe Gold Mine Case, Turkey. Water, Air, & Soil Pollution, **234** (4), 272, **2023**.
25. CICEK N., ERDOGAN M., YUCEDAG C., CETIN M. Improving the detrimental aspects of salinity in salinized soils of arid and semi-arid areas for effects of vermicompost leachate on salt stress in seedlings. Water, Air, & Soil Pollution, **233** (6), 197, **2022**.
26. KOBRAEE S. Effect of zinc, iron and manganese fertilization on concentrations of these metals in the stem and leaves of soybean and on the chlorophyll content in leaves during the reproductive development stages. Journal of Elementology, **21** (2), **2016**.
27. PINAR H., SIMSEK C., MUTLU N. Soil Zinc (Zn) Deficiency And Breeding In Plants. International Academic Research and Reviews in Agriculture, Forestry and Aquaculture Sciences, **87**, **2023**.
28. TRIPATHI D.K., SINGH S., SINGH S., MISHRA S., CHAUHAN D., DUBEY N. Micronutrients and their

diverse role in agricultural crops: advances and future prospective. Acta Physiologiae Plantarum, **37**, 1, **2015**.
29. JALEEL C.A., JAYAKUMAR K., CHANG-XING Z., AZOOZ M. Effect of soil applied cobalt on activities of antioxidant enzymes in Arachis hypogaea. Global Journal of Molecular Sciences, **3** (2), 42, **2008**.
30. KIELISZEK M., BANO I., ZARE H. A comprehensive review on selenium and its effects on human health and distribution in middle eastern countries. Biological Trace Element Research, **200** (3), 971, **2022**.
31. FERNANDES A.P., GANDIN V. Selenium compounds as therapeutic agents in cancer. Biochimica et Biophysica Acta (BBA)-General Subjects, **1850** (8), 1642, **2015**.
32. SCHIAVON M., BERTO C., MALAGOLI M., TRENTIN A., SAMBO P., PILON-SMITS E.A. Selenium biofortification in radish enhances nutritional quality via accumulation of methyl-selenocysteine and promotion of transcripts and metabolites related to glucosinolates, phenolics, and amino acids. Frontiers in Plant Science, **7**, 209375, **2016**.
33. TIAN M., XU X., LIU Y., XIE L., PAN S. Effect of Se treatment on glucosinolate metabolism and health-promoting compounds in the broccoli sprouts of three cultivars. Food chemistry, **190**, 374, **2016**.
34. THAVARAJAH P., SARKER A., MATERNE M., VANDEMARK G., SHRESTHA R., IDRISSI O., HACIKAMILOGLU O., BUCAK B., VANDENBERG A. A global survey of effects of genotype and environment on selenium concentration in lentils (*Lens culinaris* L.): Implications for nutritional fortification strategies. Food Chemistry, **125** (1), 72, **2011**.
35. NAHAR N. Response of mungbean to zinc, boron and molybdenum application. Sher-e- Bangla Agricultural University, Department of Soil Science, **2017**.
36. PINAR H., SIMSEK C., MUTLU N.J.I.A.R. Reviews in agriculture f., sciences a. Soil zinc (zn) deficiency and breeding in plants. 87, **2023**.
37. FERNANDES A.P., GANDIN V. Selenium compounds as therapeutic agents in cancer. Biochimica et Biophysica Acta (BBA)- General Subjects, **1850** (8), 1642, **2015**.
38. JALAL A., GALINDO F.S., FREITAS L.A., DA SILVA OLIVEIRA C.E., DE LIMA B.H., PEREIRA Í.T., FERRAZ G.F., DE SOUZA J.S., DA COSTA K.N., NOGUEIRA T.A.R. Yield, zinc efficiencies and biofortification of wheat with zinc sulfate application in soil and foliar nanozinc fertilisation. Crop and Pasture Science, **73**, 749, **2022**.
39. SCHIAVON M., BERTO C., MALAGOLI M., TRENTIN A., SAMBO P., DALL'ACQUA S., PILON-SMITS E.A.H. Selenium biofortification in radish enhances nutritional quality via accumulation of methyl-selenocysteine and promotion of transcripts and metabolites related to glucosinolates, phenolics, and amino acids. Frontiers in Plant Science, **7**, 1371, **2016**.
40. EL-KHATEEB M., SAKR S., HASHISH K., MAZHAR A.A., ABDEL-MAGIED H. Effect of some mineral nutrients on vegetative growth, chemical constituents, and anatomical structure of Grevillea robusta seedlings. Egyptian Journal of Chemistry, **66** (8), 191, **2023**.
41. THAVARAJAH D., RUSZKOWSKI J., VANDENBERG A. High potential for selenium biofortification of lentils (*Lens culinaris* L.). Journal of Agriculture and Food Chemistry, **56** (22), 10747, **2008**.
42. WEIR T., PERRY L., GILROY S., VIVANCO J. J. The role of root exudates in rhizosphere interactions with plants and other organisms. Annual Review of Plant Biology (errata), 57, 233, **2006**.
43. MAHMOOD I.A., IMRAN M., SARWAR M., KHAN M., SARWAR M.A., AHMED S., MALIK S.R. Growth and yield of lentil (*Lens esculenta* l.) influenced by Zn application with PGPR inoculation under rain-fed conditions. Pakistan Journal of Agricultural Research, **32** (3), 435, **2019**.
44. ZOPE V., EL ENSHASY H.A., SAYYED R. Plant growth-promoting rhizobacteria: an overview in agricultural perspectives. Plant Growth Promoting Rhizobacteria for Sustainable Stress Management: Volume 2: Rhizobacteria in Biotic Stress Management, 345, **2019**.
45. LI B., QI G., LI Y., ZHAO X.J. Microbial network and composition changes according to tobacco varieties and interferes differently in black shank disease defense. Journal of Applied Microbiology, **134** (1), lxac001, **2023**.
46. HALA K. Effect of cobalt fertilizer on growth, yield and nutrients status of faba bean (*Vicia faba* L.) plants. Agricultural and Food Sciences, **3** (9), 867, **2007**.
47. AFFILIATION S. Optimization of cobalt and nitrogen for improving seed yield, protein content and nitrogen use efficiency in mungbean. Journal of Environmental Agriculture, **2** (1), 173, **2017**.
48. LYONS G.H., GENC Y., SOOLE K., STANGOULIS J., LIU F., GRAHAM R. Selenium increases seed production in Brassica. Plant and Soil, **318**, 73, **2009**.
49. KHAN M.R., KHAN M.M. Effect of varying concentration of nickel and cobalt on the plant growth and yield of chickpea. Australian Journal of Basic and Applied Sciences, **4** (6), 1036, **2010**.
50. LONGCHAMP M., CASTREC-ROUELLE M., BIRON P., BARIAC T. Variations in the accumulation, localization and rate of metabolization of selenium in mature Zea mays plants supplied with selenite or selenate. Food Chemistry, **182**, 128, **2015**.
51. AIN Q., AKHTAR J., AMJAD M., HAQ M., SAQIB Z. Effect of enhanced nickel levels on wheat plant growth and physiology under salt stress. Communications in soil science and plant analysis, **47** (22), 2538, **2016**.
52. KANDIL H., FARID I.M., EL-MAGHRABY A. Effect of cobalt level and nitrogen source on quantity and quality of soybean plant. Journal of Basic and Applied Scientific Research, **3** (12), 185, **2013**.
53. CHATTERJEE R., BANDYOPADHYAY S. Effect of boron, molybdenum and biofertilizers on growth and yield of cowpea (*Vigna unguiculata* L. Walp.) in acid soil of eastern Himalayan region. Journal of the Saudi Society of Agricultural Sciences, **16** (4), 332, **2017**.
54. GIANNAKOULA A., THERIOS I., CHATZISSAVVIDIS C. Effect of lead and copper on photosynthetic apparatus in citrus (*Citrus aurantium* L.) plants. The role of antioxidants in oxidative damage as a response to heavy metal stress. Plants, **10** (1), 155, **2021**.
55. THAVARAJAH D., RUSZKOWSKI J., VANDENBERG A. High potential for selenium biofortification of lentils (*Lens culinaris* L.). Journal of Agricultural and Food Chemistry, **56** (22), 10747, **2008**.
56. KUMAR H., DUBEY R., MAHESHWARI D. Rhizobial genetic diversity in root nodules of Trigonella foenum-graecum cultivated in sub-himalayan region of Uttarakhand. Biocatalysis and Agricultural Biotechnology, **16**, 243, **2018**.
57. KUMAR H., DUBEY R., MAHESHWARI D.J.B. Rhizobial genetic diversity in root nodules of Trigonella

foenum-graecum cultivated in sub-himalayan region of Uttarakhand. Biotechnology, **16**, 243, **2018**.
58. EKANAYAKE L.J. Selenium on Increasing Lentil (Lens Culinaris Medikus.) Grain Yield. North Dakota State University, **2014**.
59. KANDIL H., FARID I.M., EL-MAGHRABY A. Effect of cobalt level and nitrogen source on quantity and quality of soybean plant. Agricultural and Food Sciences, **3**, (12), 185, **2013**.
60. ISLAM M.M., KARIM M.R., OLIVER M.M.H., URMI T.A., HOSSAIN M.A., HAQUE M.M. J.A. Impacts of trace element addition on lentil (*Lens culinaris* L.). Agronomy, **8**, (7), 100, **2018**.
61. TOMIĆ D., STEVOVIĆ V., MADIC M., MARJANOVIĆ M., PAVLOVIĆ N., LAZAREVIĆ Đ., PETROVIĆ M., ZORNIĆ V., KNEŽEVIĆ J. The role of cobalt in forage legumes. XXVIII savetovanje o biotehnologiji sa međunarodnim učešćem. **2023**.
62. ZOPE V., EL ENSHASY H. A., SAYYED R. Plant growth-promoting rhizobacteria: an overview in agricultural perspectives. Agricultural and Food Sciences, 345, **2019**.
63. LI B., QI G., LI Y., ZHAO X. Microbial network and composition changes according to tobacco varieties and interferes differently in black shank disease defense. Journal of Applied Microbiology, **134** (1), lxac001, **2023**.
64. JALAL A., GALINDO F.S., FREITAS L.A., DA SILVA OLIVEIRA C.E., DE LIMA B.H., PEREIRA Í.T., FERRAZ G.F., DE SOUZA J.S., DA COSTA K.N., NOGUEIRA T. Yield, zinc efficiencies and biofortification of wheat with zinc sulfate application in soil and foliar nanozinc fertilisation. Crop and Pasture Science, **73**, 749, **2022**.
65. SEREGIN I.V., KOZHEVNIKOVA A.D. Phytochelatins: Sulfur-containing metal (loid)-chelating ligands in plants. International Journal of Molecular Sciences, **24** (3), 2430, **2023**.
66. THAVARAJAH D., ABARE A., MAPA I., COYNE C.J., THAVARAJAH P., KUMAR S. Selecting lentil accessions for global selenium biofortification. Plants, **6** (3), 34, **2017**.
67. KLIMEK-KOPYRA A., BARAN A., ZAJĄC T., KULIG B. Effects of heavy metals from polluted soils on the roots and nodules formation. Bulgarian Journal of Agricultural Science, **21** (2), 295, **2015**.
68. GHASEMI Z., GHADERIAN S.M., MONTERROSO C., KIDD P.S. Improving the growth of Ni-hyperaccumulating plants in serpentine quarry tailings. International Journal of Phytoremediation, **20** (7), 699, **2018**.
69. HALA K. Effect of cobalt fertilizer on growth, yield and nutrients status of faba bean (*Vicia faba* L.) plants. Journal of Applied Scientific Research, **3** (9), 867, **2007**.
70. KLIMEK-KOPYRA A., BARAN A., ZAJĄC T., KULIG B. Effects of heavy metals from polluted soils on the roots and nodules formation. Environmental Science, **21** (2), 295, **2015**.
71. THAVARAJAH D., THAVARAJAH P., VIAL E., GEBHARDT M., LACHER C., KUMAR S., COMBS G. Will selenium increase lentil (Lens culinaris Medik) yield and seed quality? Frontiers in Plant Science, **6**, 356, **2015**.
72. MINZ A., SINHA A.K., KUMAR R., KUMAR B., DEEP K.P., KUMAR S. A review on importance of cobalt in crop growth and production. International Journal of Current Microbiology and Applied Science, **7**, 2978, **2018**.
73. HÄNSCH R., MENDEL R.R. Physiological functions of mineral micronutrients (cu, Zn, Mn, Fe, Ni, Mo, B, cl). Current Opinion in Plant Biology, **12**, (3), 259, **2009**.
74. YE-TAO T., TENG-HAO-BO D., QI-HANG W., SHI-ZHONG W., RONG-LIANG Q., ZE-BIN W., XIAO-FANG G., QI-TANG W., MEI L., TONG-BIN C. Designing cropping systems for metal-contaminated sites: a review. Pedosphere, **22** (4), 470, **2012**.
75. KUDOYAROVA G., ARKHIPOVA T., DODD I.C. Phytohormone mediation of interactions between plants and non-symbiotic growth promoting bacteria under edaphic stresses. Frontiers in Plant Science, **10**, 483140, **2019**.
76. ADILA IRAM T., TAHIR HUSSAIN A., TANVEER A., NADEEM AKBAR M., FARUKKH S., SAFDAR M.E., KAKU S. Optimization of cobalt and nitrogen for improving seed yield, protein content and nitrogen use efficiency in mungbean, **2** (1), 173, **2017**.
77. MINZ A., SINHA A.K., KUMAR R., KUMAR B., DEEP K.P., KUMAR S.B. A review on importance of cobalt in crop growth and production. International Journal of Current Microbiology and Applied Sciences, **7** (2), 2978, **2018**.
78. HÄNSCH R., MENDEL R.R. Physiological functions of mineral micronutrients (cu, Zn, Mn, Fe, Ni, Mo, B, cl). Current Opinion in Plant Biology, **12** (3), 259, **2009**.
79. HOUSHMANDFAR A., MORAGHEBI F. Effect of mixed cadmium, copper, nickel and zinc on seed germination and seedling growth of safflower. African Journal of Agricultural Research **6** (5), 1182, **2011**.
80. SAADANI O., JEBARA S.H., FATNASSI I.C., CHIBOUB M., MANNAI K., ZARRAD I., JEBARA M. Effect of Vicia faba L. var. minor and Sulla coronaria (L.) Medik associated with plant growth-promoting bacteria on lettuce cropping system and heavy metal phytoremediation under field conditions. Environmental Science and Pollution Research, **26**, 8125, **2019**.
81. ZHANG X., LAUBIE B., HOUZELOT V., PLASARI E., ECHEVARRIA G., SIMONNOT M. Increasing purity of ammonium nickel sulfate hexahydrate and production sustainability in a nickel phytomining process. Environmental Science, Chemistry, **106**, 26, **2016**.
82. HOUSHMANDFAR A., MORAGHEBI F. Effect of mixed cadmium, copper, nickel and zinc on seed germination and seedling growth of safflower. African Journal of Agricultural Research, **6** (5), 1182, **2011**.
83. BACHIEGA P., DE ALMEIDA E., SALGADO J.M., ARRUDA M.A.Z., LEHMANN E.L., MORZELLE M.C., DE CARVALHO H.W.P. Benchtop and handheld energy-dispersive X-ray fluorescence (EDXRF) as alternative for selenium concentration measurement in biofortified broccoli seedling. Food Analytical Methods, **12**, 1520, **2019**.
84. SAADANI O., JEBARA S.H., FATNASSI I.C., CHIBOUB M., MANNAI K., ZARRAD I., JEBARA M. Effect of *Vicia faba* L. var. minor and *Sulla coronaria* (L.) Medik associated with plant growth-promoting bacteria on lettuce cropping system and heavy metal phytoremediation under field conditions. Environmental Science and Pollution Research, **26**, 8125, **2019**.
85. WANG N., HATCHER D., TOEWS R., GAWALKO E. Influence of cooking and dehulling on nutritional composition of several varieties of lentils (*Lens culinaris*). LWT-Food Science and Technology, **42** (4), 842, **2009**.
86. FAGERIA N.K. The use of nutrients in crop plants. CRC press, **2016**.
87. ROYCHOUDHURY A., CHAKRABORTY S. Cobalt and molybdenum: deficiency, toxicity, and nutritional role in plant growth and development. Elsevier, **2022**.

88. WELCH R.M. Linkages between trace elements in food crops and human health. Micronutrient Deficiencies in Global Crop Production, 287, **2008**.
89. ASLAM M., MAHMOOD I., PEOPLES M., SCHWENKE G., HERRIDGE D. Contribution of chickpea nitrogen fixation to increased wheat production and soil organic fertility in rain-fed cropping. Biology and Fertility of Soils, **38**, 59, **2003**.
90. GARG N., SAROY K. Interactive effects of polyamines and arbuscular mycorrhiza in modulating plant biomass, N2 fixation, ureide, and trehalose metabolism in *Cajanus cajan* (L.) Millsp. genotypes under nickel stress. Environmental Science and Pollution Research, **27** (3), 3043, **2020**.
91. GOUFO P., MOUTINHO-PEREIRA J.M., JORGE T.F., CORREIA C.M., ANTÓNIO C., TRINDADE H. Cowpea (Vigna unguiculata L. Walp.) metabolomics: osmoprotection as a physiological strategy for drought stress resistance and improved yield. Frontiers in Plant Science, **8**, 261900, **2017**.
92. GOUFO P., MOUTINHO-PEREIRA J.M., JORGE T.F., CORREIA C.M., OLIVEIRA M.R., ROSA E.A., ANTÓNIO C., TRINDADE H. Cowpea (*Vigna unguiculata* L. Walp.) metabolomics: osmoprotection as a physiological strategy for drought stress resistance and improved yield. Frontiers in Plant Science, **8**, 586, **2017**.
93. JAYAKUMAR K., JALEEL C.A., AZOOZ M. Impact of cobalt on germination and seedling growth of Eleusine coracana L. and Oryza sativa L. under hydroponic culture. Global Journal of Molecular Sciences, **3** (1), 18, **2008**.
94. KHATAB A. Improving growth, yield and nutrient uptake of Faba bean (*Viciafaba* l.) By inoculation with Mycorrhizay and foliar appliction of cobalt under saline irrigation water on a calcareous soil. Journal of Soil Sciences and Agricultural Engineering, **7** (3), 249, **2016**.
95. COLLINS R.N., BAKKAUS E., CARRIÈRE M., KHODJA H., PROUX O., MOREL J.-L., GOUGET B. Uptake, localization, and speciation of cobalt in *Triticum aestivum* L. (wheat) and *Lycopersicon esculentum* M. (tomato). Environmental Science & Technology, **44** (8), 2904, **2010**.
96. ASAGBA S., APIAMU A., ENOKPE F. Effects of nickel toxicity on the indices of germination and $Ca^{2+}$ ATPase activity in cowpea plant (*Vigna unguiculata*). Journal of Applied Sciences and Environmental Management, **23** (6), 1147, **2019**.
97. KHAN M.I.R., NAZIR F., ASGHER M., PER T.S., KHAN N.A. Selenium and sulfur influence ethylene formation and alleviate cadmium-induced oxidative stress by improving proline and glutathione production in wheat. Journal of Plant Physiology, **173**, 9, **2015**.
98. ASATI A., PICHHODE M., NIKHIL K. Effect of heavy metals on plants: an overview. International Journal of Application or Innovation in Engineering & Management, **5** (3), 56, **2016**.
99. YANG B., SHU W., YE Z., LAN C., WONG M.H. Growth and metal accumulation in vetiver and two Sesbania species on lead/zinc mine tailings. Chemosphere, **52** (9), 1593, **2003**.
100. ANDERSON J.P., DOMSCH K.H. A physiological method for the quantitative measurement of microbial biomass in soils. Soil Biology and Biochemistry, **10** (3), 215, **1978**.
101. VELÁZQUEZ E., CARRO L., FLORES-FÉLIX J.D., MENÉNDEZ E., RAMÍREZ-BAHENA M.-H., PEIX A. Bacteria-inducing legume nodules involved in the improvement of plant growth, health and nutrition. Microbiome in Plant Health and Disease: Challenges and Opportunities, 79, **2019**.
102. ASATI A., PICHHODE M., NIKHIL K. Effect of heavy metals on plants: an overview. International Journal of application or Innovation in Engineering & Management, **5** (3), 56, **2016**.
103. YANG B., SHU W., YE Z., LAN C., WONG M. H. Growth and metal accumulation in vetiver and two Sesbania species on lead/zinc mine tailings. Chemosfere, **52** (9), 1593, **2003**.
104. COLLINS R.N., BAKKAUS E., CARRIÈRE M., KHODJA H., PROUX O., MOREL J.-L., GOUGET B. Uptake, localization, and speciation of cobalt in *Triticum aestivum* L. (wheat) and *Lycopersicon esculentum* M. (tomato). Environmental Science and Technology, **44** (8), 2904, **2010**.
105. ASAGBA S., APIAMU A., ENOKPE F.E. Effects of nickel toxicity on the indices of germination and $Ca^{2+}$ ATPase activity in cowpea plant (*Vigna unguiculata*). Journal of Applied Sciences and Environmental Management, **23**, (6), 1147, **2019**.
106. KHATAB A. Improving growth, yield and nutrient uptake of Faba bean (*Viciafaba* l.) By inoculation with mycorrhizay and foliar appliction of cobalt under saline irrigation water on a calcareous soil. Agricultural and Food Sciences, **7** (3), 249, **2016**.
107. LAL K., KUMAR R., SHRIVASTAV S.P., KUMAR A., SINGH Y. Genetic variability, character association and path analysis of seed yield and its contributing traits in field pea (*Pisum sativum* L. var. arvense). International Journal of Current Microbiology Applied Sciences, **7** (6), 1815, **2018**.
108. ANJUM K., QADIR I., AHMAD H.M., SAHER M., AFZAL S., RASOOL F., NOUMAN W., YOUSAF M.T.B., ALI A. Economic rotation of Dalbergia sissoo in Tehsil Khushab. Journal of Agricultural Research, **55**, (3), 537, **2017**.
109. CETIN M., ABO AISHA A.E.S. Variation of Al concentrations depending on the growing environment in some indoor plants that used in architectural designs. Environmental Science and Pollution Research, **30** (7), 18748, **2023**.
110. BOZDOGAN SERT E., TURKMEN M., CETIN M. Heavy metal accumulation in rosemary leaves and stems exposed to traffic-related pollution near Adana-İskenderun Highway (Hatay, Turkey). Environmental Monitoring and Assessment, **191**, 1, **2019**.