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Phytoextraction Potential of Flax Grown on Multimetal Contaminated Soils: A field Experiment

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Abstract: A two-year field experiment (2022–2023) was carried out to investigate the impact of different sowing periods (spring and winter) and nitrogen levels (0, 30, 60 kg/ha) on the phytoextraction potential of flax. The study site is characterized by high concentrations of heavy metals (Cd, Ni, Cu, Pb, Zn) and belongs to the Mediterranean climate type. Flax (var. Calista) was sown in the spring of 2022 and in the winter of 2023, following a split plot experimental design with three replications, and applying low-input practices. Results showed that spring-sown flax produced shorter but thicker plants with significantly higher biomass (5.27 tn ha^{-1}) when treated with 30 kg ha^{-1} N compared to winter-sown (2.30 tn ha^{-1}) when treated with 60 kg ha^{-1} N. The concentration of contaminants in the aerial biomass varied according to metal type and sowing period. The higher biomass production in spring resulted in a higher quantity of heavy metals being removed from the soil, making flax a promising crop for phytoextraction purposes, especially in soils contaminated with multiple heavy metals.

Keywords: heavy metals; *Linum usitatissimum*; nitrogen fertilization; phytoremediation; sowing period; uptake

1. Introduction

The increasing industrialization and intensification of agriculture during the last century resulted in an increased burden of soils globally with several types of pollutants. The most common soil contaminants found at sites in the European Union (EU) are heavy metals [1]. An approximate estimate of 2.5 million possibly polluted sites is projected for the entire European region; about 14% of them (340.000 sites) are believed to be hazardous and necessitate cleanup efforts [1]. Therefore, it is crucial to carry out remediation measures to reduce land contamination. The use of chemical and physical methods for soil decontamination includes soil washing, stabilization, thermal desorption, excavation and landfill, electric field application, etc. [2,3]. However, these methods have limitations, such as high cost, inefficiency when dealing with low concentrations of pollutants, and changes to the physiochemical characteristics of the soil. In recent decades, a cost-effective, environmentally friendly, and publicly acceptable approach to soil remediation, has emerged, known as phytoremediation. This method uses plants to cleanse the environment by extracting, accumulating, stabilizing, and detoxifying contaminants from the substrate (soil, air, and water) through physical, chemical, or biological processes [4]. Even though phytoremediation has been scientifically proven to be effective in addressing contaminants, it still faces significant challenges, as it is time-consuming and potentially harmful on living organisms due to biomagnification. Nevertheless, these drawbacks can be addressed by using non-edible commercial plants characterized by rapid growth rates and

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low maintenance requirements. Bast fiber crops emerge as promising candidates for phytoremediation [5,6].

Flax (*Linum usitatissimum* L.) is a well known fiber crop with high potential for effective utilization in phytoremediation. It grows best in moderate climates, especially in regions receiving an annual precipitation of at least 600-650 mm, with a minimum of 110-150 mm of rain during the vegetation period [7]. Additionally, unfavorable climatic conditions, such as strong winds, excessive rainfall, high temperatures, and water shortage, can negatively affect the growth and development of flax crops [8]. However, there are many varieties and genotypes that are suitable for various regions and weather conditions all over the world [9]. In addition, one of the most important agronomic factors of flax productivity is nitrogen fertilization [10]. Nitrogen (N) has a critical impact on fiber content, fiber length, and stem diameter in flax [7]. While it is essential for growth, excessive doses of nitrogen can result in stem thickening and a reduction in fiber strength [7].

The tolerance of flax fiber to toxic pollutants in soil and its capability to accumulate heavy metals has been mentioned in several studies [2,6,11-15]. Flax is a crop that is well known for its large scale agronomic and harvesting practices, as well as for its multiple industrial applications. The harvested fiber could be used in manufacturing biomaterials, contributing to industries such as pulp and paper, textile, furniture, and chemicals [5,16-18].

The Lavreotiki Peninsula is an area of high geological and archaeological importance. Situated approximately 60 km southeast of Athens, Greece, it is well known for its long history and mining activities that have spanned for more than 5,000 years. Mining activities in the region predominantly focused on argentiferous galena, an important source of profit for ancient Aegean civilizations, likely begun prior to 3500 BC [19]. Records of silver (Ag) production date back to the 7th century BC, making Lavreotiki historically important for its role in silver production, especially for the Athenian drachma. These mining activities are estimated to have produced approximately 3.500 tons of silver and around of 1.400.000 tons of lead (Pb), with a significant part, roughly 70%, extracted during the 5th and 4th centuries BC [20]. The decline of mining operations began in the 3rd century BC, eventually pause altogether by the 1st century BC. Modern-era mining activities were restarted in the mid- 19th century and continued until 1980 [21]. In 1900, Lavrion smelters involved 3% of the global lead production [20]. The history of mining, ore processing and smelting has produced potentially harmful residues scattered throughout the urban and suburban areas of the modern Lavrion city [19].

The main objective of this study is to assess the phytoextraction potential of flax (var. Calista) under field conditions, using a multi- heavy metal contaminated field in Lavreotiki Peninsula. Additionally, the study aims to explore the impacts of sowing period (spring and winter) and applied nitrogen fertilization rates (0 kg N ha^{-1}, 30 kg N ha^{-1}, and 60 kg N ha^{-1}) on the growth, development, and phytoextraction capacity of flax plants.

2. Results

2.1. Soil Characterization

Soil chemical and physical properties of the sample are listed in Table 1. As per USDA soil texture classifications, the soil texture was categorized as slit clay. The soil exhibited a medium level of total nitrogen, a low level of available P and a high level of available K. Organic matter content was slightly low.

Table 1. Soil physical and chemical properties of the experimental field.

Properties	
pH	8.2 ± 0
Organic matter (%)	2.75 ± 0
CEC (%)	28.9 ± 0.99
$CaCO_3$ (%)	5.30 ± 0.30
Conductivity (μS cm^{-1})	220.5 ± 9.20
Total N (%)	0.2 ± 0

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Available P (mg kg^{-1})	6.8 ± 0.21
Available K (mg kg^{-1})	564.7 ± 57.30
Mechanical analysis	
Clay (%)	36.0 ± 2.83
Silt (%)	47.0 ± 2.83
Sand (%)	17.0 ± 0
Texture	Silt-clay

The results of the total and bioavailable heavy metal concentrations indicate a significant level of heavy metal contamination in the soil of the study area (Table 2).

Table 2. Total and bioavailable content of Cd, Ni, Cu, Pb and Zn (mg kg^{-1}) in soil of experimental field.

Total Content (mg /kg^{-1})	
Cd	6.45 ± 0.2
Ni	114.5 ± 14.8
Cu	149.0 ± 11.3
Pb	3279.5 ± 362.7
Zn	2238.0 ± 148.5
Bioavailable Content (mg /kg^{-1})	
Cd	1.95 ± 0.1
Ni	0.8 ± 0
Pb	525.5 ± 77.10
Zn	79.5± 6.4

2.2. Phenological and Agronomical Traits

Flax showed a shorter growth cycle, from sowing to harvest, when sown in spring compared to the winter sowing, with a cycle length of 112 days and 177 days, respectively. The growth and development of flax were significantly influenced by the cultivation period (Table 3). Climatic data, concerning the experimental field are available in Appendix A.

Table 3. Agronomical characterization of tested flax cultivar.

Agricultural Period	Nitrogen Level	Height (cm)	Shoot Diameter (mm)	No. of Branches
Spring cultivation (2022)	N0	59.02± 12.0 ^{a}	3.74± 1.0 ^{a}	0.50± 0.8 ^{a}
	N1	50.73± 6.8 ^{a}	3.62± 0.9 ^{a}	0± 0 ^{a}
	N2	54.58± 6.7 ^{a}	4.53± 0.8 ^{a}	1.00± 1.3 ^{a}
Winter cultivation (2023)	N0	76.71± 7.7 ^{a}	2.07± 0.6 ^{a}	1.50± 1.0 ^{a}
	N1	77.44± 9.1 ^{a}	2.29± 0.5 ^{ab}	1.61± 0.8 ^{a}
	N2	81.39± 6.2 ^{b}	2.48± 0.5 ^{b}	1.61± 0.6 ^{a}

± standard deviation, $p<0.05$, ^{a,b} varying letters indicate significant differences across treatments for each cultivation period with 95% confidence level.

Notably, significant height differences among nitrogen levels were detected only in the winter sowing of 2023, specifically in N2 nitrogen level compared with control and N1 treated plants. Two-way ANOVA did not reveal significant variations in the interaction between cultivation period and nitrogen level. During spring cultivation, untreated plots showed higher plants compared to treated plants but with no significant differences, as presented in Table 3. In contrast, during winter cultivation, plant height showed a linear increase with increasing nitrogen concentrations (Table 3). Significant height differences among nitrogen levels were observed in N2 nitrogen level compared

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with control and N1 treated plants. An elevation in nitrogen levels was associated with an increase in the shoot diameter of flax during both cultivation periods (Table 3). At the end of first cultivation year there was an increase tendency but with no significant differences, while at the end of the second cultivation year there was a statistically significant difference at N2 nitrogen level. However, two-way ANOVA showed significant differences in shoot diameter based on applied nitrogen levels and cultivation periods, with no significant interaction between these factors. The evaluation of stem branching did not show significant differences between nitrogen levels and cultivation periods, with the cultivation period identified as the influencing factor. Non-significant differences in stem branching were observed in spring sowing, and similar results were obtained during winter cultivation (Table 3).

2.3. Biomass Yield

Table 4 presents flax dry weights (tn ha^{-1}) influenced by cultivation period and nitrogen levels for 2022 and 2023. Two-way ANOVA showed significant effects from both the cultivation period and the interaction between the cultivation period and nitrogen levels. In spring, N1 treatment increases dry biomass compared to untreated plots but with no significant differences, while N2 treatment gave significantly lower yields compared to N1 (Table 4). At the end of the second cultivation year, final biomass was higher under N2 treatment, compared to N1 and the control, although no significant statistical differences were observed (Table 4).

Table 4. Biomass yields for the two cultivation periods.

Agricultural period	Nitrogen level	Biomass yields (tn ha^{-1})
Spring cultivation (2022)	N0	3.81± 0.9^{ab}
	N1	5.27± 1.0^{b}
	N2	2.88± 0.5^{a}
Winter cultivation (2023)	N0	2.21± 0.6^{a}
	N1	2.09± 0.7^{a}
	N2	2.30± 0.6^{a}

± standard deviation, $p<0.05$, ^{a,b} varying letters indicate significant differences across treatments for each cultivation period with 95% confidence level.

2.4. Heavy Metal Concentration and Uptake

The levels of cadmium (Cd), nickel (Ni), copper (Cu), lead (Pb), and zinc (Zn) in flax aerial biomass for both cultivation periods, and under the different nitrogen treatments, are presented in Tables 5 and 6.

Table 5. Heavy metals concentrations (mg kg^{-1}) in the above-ground biomass of flax for the two cultivation periods.

Agricultural period	Nitrogen level	Cd (mg kg^{-1})	Ni (mg kg^{-1})	Cu (mg kg^{-1})	Pb (mg kg^{-1})	Zn (mg kg^{-1})
Spring cultivation (2022)	N0	8.46± 0.9^{a}	<D.L.	6.35±1.1^{a}	18.15±3.9^{a}	51.85±11.1^{b}
	N1	6.95±0.8^{a}	0.22±0.1	5.39±1.5^{a}	16.04±2.4^{a}	40.06±5.1^{ab}
	N2	7.14±1.2^{a}	<D.L.	5.14±1.5^{a}	17.87±0.9^{a}	35.16±1.0^{a}
Winter cultivation (2023)	N0	6.73±1.0^{a}	<D.L.	6.76±0.5^{a}	7.21±0.6^{a}	48.60±11.7^{a}
	N1	5.82±1.2^{a}	<D.L.	6.47±0.3^{a}	7.48±0.7^{a}	50.89±17.0^{a}
	N2	5.84±1.9^{a}	<D.L.	7.21±0.1^{a}	13.02±4.9^{b}	49.00±8.8^{a}

± standard deviation, $p<0.05$, ^{a,b} varying letters indicate significant differences across treatments for each cultivation period with 95% confidence level.

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Table 6. Heavy metals uptake (g ha^{-1}) of flax for the two cultivation periods.

Agricultural Period	Nitrogen level	Cd (g ha^{-1})	Ni (g ha^{-1})	Cu (g ha^{-1})	Pb (g ha^{-1})	Zn (g ha^{-1})
Spring cultivation (2022)	N0	31.81±6.1^{b}	<D.L.	24.53±9.2^{a}	71.28±32.5^{a}	190.68±8.9^{b}
	N1	36.15±3.1^{b}	1.10±0.3	27.47±2.5^{a}	85.70±27.7^{a}	212.10±56.1^{b}
	N2	20.38±3.8^{a}	<D.L.	15.30±6.9^{a}	51.69±10.8^{a}	101.38±18.5^{a}
Winter cultivation (2023)	N0	14.59±2.8^{a}	<D.L.	14.81±3.1^{a}	15.72±2.9^{a}	103.43±11.3^{a}
	N1	12.51±6.4^{a}	<D.L.	13.48±4.6^{a}	15.29±3.6^{a}	109.15±63.2^{a}
	N2	12.78±2.4^{a}	<D.L.	16.61±4.2^{a}	28.10±2.1^{b}	110.22±21.2^{a}

± standard deviation, $p<0.05$, ^{a,b} varying letters indicate significant differences across treatments for each cultivation period with 95% confidence level.

2.4.1. Cd Concentration and Uptake

In 2022 and 2023, the cadmium concentration in the aerial parts of flax did not show significant differences among the nitrogen treatments (N0, N1, and N2) for both cultivation periods (Table 5). Two-way ANOVA revealed a significant difference in the cultivation period, while no significant difference was observed in the interaction between the cultivation period and applied nitrogen levels. However, the uptake capacity of flax did not follow the same pattern as heavy metal concentration (Table 6). In 2022, there was a significant difference in uptake capacity among nitrogen treatments, with N0 and N1 reaching higher values, compared to N2. In 2023, the untreated plots recorded the highest mean value, while no significant differences were observed among nitrogen treatments. Two-way ANOVA indicated significant differences in cultivation period, nitrogen levels, and the interaction between these factors.

2.4.2. Ni Concentration and Uptake

Nickel accumulation in flax plants in 2022 was below the detection limit for control and N2 treatment levels, while in N1 treatment gave a concentration of 1.10 mg kg-1 (Table 5). In 2023, nickel concentration was below the detection limit in all tested treatments (Table 5). Nickel uptake capacity of flax follows the same pattern (Table 6). Two-way ANOVA for accumulation and uptake revealed significant differences in cultivation period, nitrogen levels, and the interaction between these factors.

2.4.3. Cu Concentration and Uptake

Copper concentrations during the spring cultivation period were decreased as the nitrogen level increased, although no significant differences were observed (Table 5). In the winter cultivation, copper concentration insignificantly increased in plots treated with N2 level of nitrogen compared to control and N1 treated plots (Table 5). Copper accumulation (g ha^{-1}) increased when flax was treated with N1 nitrogen level in the spring season compared to N0 and N2 treatments, but no significant differences were detected among treatments (Table 6). Nitrogen affected flax differently in the winter season, since increased copper content in the harvestable parts was detected in N2 treated plots, although no significant differences were observed (Table 6). A two-way ANOVA analysis for copper concentration and uptake showed a significant difference between the cultivation periods, while no significant differences were observed in the interaction between the cultivation period and nitrogen level.

2.4.4. Pb concentration and Uptake

In 2022, no significant differences were detected in lead concentration in flax tissues; higher but not significant concentrations were measured in the control plots, followed by N2 and N1 treatments (Table 5). In 2023, lead concentration increased linearly with the increasing applied nitrogen; the N2 treated plants concentrated lead in significantly higher amounts compared to N0 and N1 (Table 5). Two-way ANOVA indicated a significant difference in the cultivation period, while no significant

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difference was observed in the interaction between the two tested factors – cultivation period and nitrogen level. In 2022, the uptake capacity of lead in flax showed an increase at the low nitrogen level compared to the control and N2 treated plants; however, no significant differences were observed among treatments (Table 6). In the second cultivation period in 2023, significantly higher values were observed under the high nitrogen treatment (Table 6). In two-way ANOVA, only the cultivation period showed a significant difference, while no significant differences were observed in the interaction between the cultivation period and nitrogen level.

2.4.5. Zn Concentration and Uptake

During spring cultivation, a significant decreasing tendency in zinc concentration in its upper tissues was noted as nitrogen-treated levels increased (Table 5). The highest values were recorded in the control plots, followed by N1 and N2 (Table 5). In winter flax cultivation in 2023 the concentrations of zinc did not differ significantly among treated and control plants (Table 5). Neither cultivation period nor nitrogen level showed a significant difference in the two-way ANOVA test, and no significant differences were observed in the interaction between factors. In 2022, zinc uptake capacity was significantly higher in N0 and N1 treatment compared to N2 treatment (Table 6). In 2023, in zinc accumulation no significant differences were observed among treatments (Table 6). The two-way ANOVA test showed significant differences in cultivation period and in the interaction between the two tested factors, i.e. cultivation period and nitrogen level.

3. Discussion

Our study reveals that nitrogen fertilization significantly affects flax productivity, with spring-sown flax responding better to 30 kg N ha^{-1}, producing shorter but thicker plants and higher biomass compared to winter-sown flax treated with 60 kg N ha^{-1}. Flax shows a positive response to nitrogen fertilization and increased soil concentrations of heavy metals, making it a promising candidate for phytoremediation.

Previous researches underline the complex interaction of factors that impact flax development, such as residual soil nitrogen, soil type, flax cultivar, climate and moisture conditions, and growing cycle duration [10,14,22-26]. Furthermore, the key roles of plant density, climatic conditions, and nitrogen levels in shaping stem branching in flaxseed have been emphasized in various studies [25-30]. Understanding the importance of these factors is crucial when evaluating flax branching patterns, as they significantly affect plant architecture and yield. Nitrogen is an important factor in the growth of flax, contributing to both fiber content and stem diameter [7,10]. Its critical role points out its significance as a key nutrient for flax cultivation, affecting the quality of fiber and the structural characteristics of the plant. Moreover, water availability during growth development appears as a critical factor affecting shoot thickness [22,31]. Additionally, during flowering and seed-filling stages, adequate water has been associated with increased biomass yields [8]. This information underlines the relationship of water supply and growth stages, influencing flax crop productivity.

The observed variations in the growth of fiber flax in our experimental field can be due to the presence of elevated concentrations of heavy metals. Heavy metals can have both direct and indirect effects on plant growth, and their impact depends on the type of metal, its concentration, and the specific plant species [32-34]. [35], have previously linked heavy metal exposure to reduced plant growth, suggesting a possible explanation for the observed growth variations. Heavy metals can have various of detrimental effects on plants growth by interrupting nutrient uptake, causing cellular damage, inhibiting enzymes and interfering with various physiological processes. It is important to note that there is limited research on the direct effects of heavy metals on fiber flax in real field conditions. Commercially cultivated fiber flax typically has an average height ranging from 80 to 150 cm [7]. Studies conducted on fiber flax grown on a sand substrate containing 0.1 μM cadmium showed an average height of 76 cm, compared to the control with a height of 115 cm [13]. Another pot experiment with a cadmium concentration of 2×10^{-4} M resulted in fiber flax reaching up to 25 cm, compared with the control height of up to 50 cm [36]. Additionally, a pot study using soil artificially spiked with copper (0, 200, 400, 600 mg kg^{-1}) revealed a linear decrease in average height

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and dry weight (89 cm, 88 cm, 83 cm, and 60 cm, respectively) as the copper content in the soil increased [2]. [6], in pot experiment that conducted with three different level of cadmium, nickel, lead and antimony in three flax cultivars, concluded that as the dose of the metal and antimony increased, the growth characteristics of the varieties decreased. The mean values of the height recorded in our study align with those reported in other field non-polluted experimental studies, resulting similar average height values for flaxseed [25,27,37]. [25] highlighted the aspect of the growing cycle duration, reporting taller linseed plants (62.9 cm) during autumn sowings compared to spring sowings (55.5 cm) in a semi-arid Mediterranean environment. This difference was attributed to the impact of a shorter growing cycle during spring sowings. These results are in accordance with the results of this paper. In contrast to previous investigations that established a linear correlation between nitrogen levels and height in flaxseed [37-40], our results at the spring cultivation vary, giving no significant differences between treated and control plants, while winter cultivation results were in accordance with the findings of the other researchers.

Optimal moisture availability in the root zone results thicker shoot diameters by increasing nutrient uptake and translocation, affecting plant growth and development [31]. Moreover, optimal soil moisture availability is related to the deposition of additional cellulosic layers on the primary wall, promoting the development of a secondary cell wall, a process proposed by [22], that likely resulted thicker shoot diameter. In contrast, low temperatures and water shortages during the winter cultivation period may negatively affect shoot thickness. Elevated nitrogen levels specifically influence the flax variety, resulting in more branches during both spring and winter cultivation. In spring cultivation, higher density leads to fewer branches, while winter cultivation characterized with lower density but with a higher number of branches per plant. These findings align with [30], suggesting that linseed, is characterized by great phenotypic plasticity with a considerable adaptability to changes in spacing. The increased stem branching in our fiber flax cultivation agrees with [26], who noted that higher nitrogen soil concentrations were associated with an increased number of stem branches in golden flaxseed. Additionally, flax varieties considered for fiber production typically exhibit less branching, featuring thinner straw and lacking sub-stems [7]. The study flax variety (var. Calista), classified as a fiber flax variety, may explain its comparatively lower results compared to other studies using flaxseed.

The main factors that affect the biomass productivity of flax are nitrogen fertilization, climatic conditions, and water availability especially during the flowering and seed filling stage and elevated concentration of heavy metals and metalloids [6-8,10]. Nitrogen fertilizer plays a crucial role in promoting plant growth and productivity across various crops, with studies suggesting increased nitrogen levels lead to elevated biomass [37,38,41,42], although excessive nitrogen application, may reduce total dry matter in flaxseed [10,43]. Climatic conditions, especially wet and cold soil in spring, negatively impact flax yield by blocking plant emergence [8,28]. A water treatment of at least 120 mm during flowering and seed filling stages in flaxseed (May to June) results in higher yields [8]. The presence of heavy metals and metalloids seems to have no visible impact on crop development and productivity when compared with other studies in unpolluted fields. This observation aligns with the findings of [11], suggesting that in a 2-year field experiment with flax, hemp and cotton in a mining site with increased cadmium, copper, zinc and lead concentrations, heavy metals did not affect crops' development and productivity. Our results point out that the flax variety Calista, treated with water during spring cultivation, yields increased biomass compared to winter cultivation without water supply. The preferred nitrogen rate appears to be N1 at 30 kg N ha^{-1}, resulting in higher yields in spring compared to N0 and N2, with no significant differences observed during winter.

The concentration and accumulation in the vegetative organs of flax differed between the two cultivation periods. Cadmium is a metal element that is not essential for plant metabolism and can be toxic even at low concentrations. It tends to accumulate mainly in roots rather than shoots, with toxicity that leads to its translocation to shoots as a defense mechanism against harmful effects on roots [32,33]. In flax, studies have shown that cadmium is mainly concentrated in roots, followed by shoots and, to a lower extent, seeds [11,12,45]. Reduced concentrations may result from the antagonistic effects with lead and zinc, as both elements interact with cadmium, decrease its uptake

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while increasing their own intake [32]. During spring cultivation with water treatment, higher concentrations of cadmium were measured, aligning with previous research [14,46], relating increased cadmium concentrations in flax with greater precipitation during the growing season, indicating higher cadmium movement in response to increased moisture availability. Nitrogen treatments did not seem to increase cadmium concentration in flax aboveground biomass in both cultivation periods, although they did increase cadmium accumulation (g ha^{-1}) in the N1 treatment during spring cultivation. Our findings parallel those of [47], who reported that nitrogen treatment reduced cadmium content in above and below-ground biomass of perennial and annual herbs but significantly increased cadmium accumulation. The concentrations of cadmium resulted from our study were generally lower when compared to those reported by [11], except for N0 in the spring season. The observed differences could be attributed to the heterogeneity of the soil in the field, which may vary between plots and lead to variations in metal concentrations.

Nickel keeps a crucial role as a micronutrient in plant biological functions, but it might be toxic at higher concentrations, while remaining essential for plant growth at lower levels [48,49]. Nickel mobility in soil is influenced by pH, soil properties, and initial metal concentration [50]. Limited data exist on nickel accumulation in flax plants. In our field, the bioavailable concentration of nickel was low (0.8 ± 0), and nickel in aboveground flax biomass remained below the detection limit in both cultivation periods, indicating an absence of nickel accumulation. Notably, the N1 treatment was the only one yielding detectable results in the studied flax variety. The low bioavailable nickel concentration in the soil and the fact that its origin is geogenic and not anthropogenic in Lavreotiki area may explain the absence of nickel content in the flax tissues.

Copper plays vital roles in various physiological and biochemical reactions within plants, establishing its status as an essential nutrient. However, excessive copper levels can lead to significant toxicity, causing disruptions in bio-physiochemical processes such as growth, nutrient and water uptake, photosynthesis, root development, and leaf expansion [51]. Copper is mainly accumulated by plants through their root system, and the transfer of Cu from soil to plant varies under different soil conditions [51]. Critical soil parameters, including soil pH and organic matter (OM), play a crucial role in controlling copper adsorption/desorption, mobility, and bioavailability in soil, influencing its uptake by plant roots [51,52]. Nitrogen application during the spring season led to a decrease in copper content, contrary to the winter season where elevated contents were observed in highly nitrogen-treated plots. [47], suggested that nitrogen and phosphorus fertilizers may reduce copper content in plants while increasing its uptake. The copper concentrations in our study closely align with those reported by [11]. Furthermore, climatic conditions and irrigation treatments during cultivation may also affect copper content in flax plant tissues. These findings underscore the complex interactions between copper, soil conditions, and the agricultural practices used.

Based on the findings of [53], even at low concentrations, lead and cadmium can cause significant harm to plants, even though they are toxic and non- essential elements. In the study by [12], the bioremediation potential of flax under different concentrations of lead, cadmium, and zinc was tested. They found that there is a positive relationship between the increase in metal concentrations in the soil and the uptake of metals by flax plants. Our study differs from these findings, specifically for lead, which has a higher total and bioavailable content. Despite this, the flax tissues did not show a corresponding increase in this metal, suggesting a potential antagonistic effect with zinc, known to reduce lead uptake [32]. Lead in flax tends to be concentrated in the aboveground parts, as observed by [12], or mostly in roots with lower quantities in stems, as reported by [11]. The application of nitrogen fertilizer caused a decrease in lead content in flax tissues in the spring period, but an increase during the winter period, particularly in the high nitrogen treatment. [47], observed a negative impact of nitrogen application on the concentration of lead. However, the concentrations resulted from our study are lower than those reported by [11]. The effect of nitrogen application on flax plants seems to be influenced by climatic conditions [10], and additionally, the plant genotype appears as an important factor in the distribution and accumulation of heavy metals [11, 12].

Zinc serves a crucial role as an essential macronutrient for plant growth [54]. However, elevated concentrations of zinc can lead to toxicity, [55]. Plants tend to accumulate zinc in their aerial parts, which is why leaves are typically where initial signs of toxicity shown [56]. [11] reported that zinc in flaxseed, cultivated in an industrially polluted region, tends to concentrate in higher quantities in roots than in stems. In contrast, [12], in a pot experiment with varying zinc concentrations (400, 800, and 1000 mg kg^{-1} soil), found that zinc in flax mainly concentrated in aboveground parts. The use of nitrogen fertilizer did not improve zinc content in flax tissues during spring cultivation, but a small, insignificant increase was observed, as suggested by our results during winter cultivation. The concentrations observed in our study are lower than those reported by [11]. The observed decrease in zinc content due to nitrogen application aligns with the findings of [46], who reported a lower zinc concentration in the aboveground parts of flaxseed with nitrogen fertilization. [46], further suggested that such a reduction could be attributed to the dilution of absorbed zinc due to increased biomass accumulation.

The fiber flax variety we tested is tolerant to cadmium, nickel, copper, lead and zinc, and has the capacity to accumulate and absorb these metals in aboveground biomass in the order given: Zn > Pb > Cd > Cu > Ni, aligning with previous studies [11,12]. Climate conditions have the biggest impact on the performance of the studied flax variety. Flax plants may not allow quick remediation of substantially metal- polluted sites, however the goal of flax cultivation is to gradually decrease the heavy metal content [57]. Although flax produces less biomass than some other crops, it has the advantage of using all of the harvested product, and this can be used in textile sector, eco- building, or to make composite furniture or automobile parts [11].

4. Materials and Methods

4.1. Soil Analysis

To determine the extent of the field heavy metal contamination, soil samples were collected from two sampling points and the physical and chemical properties, as well as the content of heavy metals were determined. Samples were taken from a depth of 0 to 30 cm, homogenized, air-dried and passed through a 2-cm sieve. The physical and chemical properties were determined according to standard procedures described by FAO soil protocol [58]. Soil pH and conductivity were determined in 1:1 soil/ distilled water suspensions after 1 hour with pH and conductivity electrodes. Organic matter content was measured by the Walkley-Black method [59]. Total and bioavailable heavy metal concentrations (i.e., cadmium, nickel, copper, lead, and zinc) in soil were determined using *aqua regia* digestion [60] and DTPA extraction [61] respectively, and quantified by an ICP-OES (Model PerkinElmer, Optimal Emission Spectrometer 8000). Soil samples of 0.5 g (dry weight) were digested using a microwave oven (Model speedwave Entry DAP-60). The temperature program was as follows: 10 min at 140 °C (power 90W), 5 min at 140 °C and 15 min at 75 °C. The resulted solutions were cooled and then the samples were centrifuged for 10 min at 2500 rpm and were filtered through Whatman Grade 1 Qualitative Filter Paper with a pore size of 20-25 μm. Subsequently, the samples were filled up to 50 ml using high purity water.

4.2. Agronomic Practices and Experimental Set Up

In 2022, a spring sowing took place on March 28, and the plants were harvested on July 18 of the same year. The winter sowing took place on December 7, 2022, and the plants were harvested on June 1, 2023. During each growing season, the Flax Council of Canada guidelines were followed for tracking the development stages of plants, which included emergence, stem elongation, flowering and plant maturity (harvest) of the flax plants [62]. Cycle length was determined as the number of days from sowing to harvest.

Before sowing, a basic fertilization was applied to the field adjusted to the results of soil analyses (Table 5); more specifically, a 16-20-0 fertilizer was used in a quantity of 350 kg ha^{-1}. Flax (var. Calista) seeds were sown in nine plots of 30 m^2 each, and in a density of 100 kg ha^{-1}. In addition, three levels of nitrogen fertilization in the form ammonium sulfate ($(NH4)_2SO_4$) were applied during the phase of stem elongation (BBCH 5) for both seasons. The applied nitrogen level was:

(i) 0 kg N ha^{-1}, which referred to as N0

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(ii) 30 kg N ha-1, which referred to as N1

(iii) 60 kg N ha-1, which referred to as N2

The single factor experimental design with three replications for each nitrogen treatment was the experimental design used in both seasons.

Due to late sowing in March, low rainfall, and high temperatures, flax cultivation required supplemental water during spring (see Appendix A). Throughout the entire cultivation period, a total of 12 irrigations were applied. Six irrigation treatments were conducted from sowing (BBCH 0) to the stage of stem elongation (BBCH 5), until late April. In early May, an additional two irrigation treatments were conducted. Two more irrigation treatments were applied at the beginning and end of June (flowering stage), with two further treatments in July as the seed-filling stage begun. The total amount of water applied was 276 mm.

4.3. Plants Sampling and Measurements

When the plants reached full maturity (BBCH 12), an evaluation was conducted on six randomly selected plants per plot to determine the plant height, the stem diameter (using a digital caliper), the number of branches, and the fresh and dry weights of the above-ground plant parts. For the determination of heavy metal contents, each plant was separated into distinct parts, specifically stems and fruits. A careful washing process was carried out, which included rinsing the parts thoroughly with tap water, deionized water and then high-purity water. The plant parts were then dried in an oven at 70°C for 48 hours, ground in a cross-hammer beater mill and sieved through a 1-mm sieve.

The digestion of plant samples was conducted using a microwave oven (Speedwave Entry DAP-60 model) following a temperature program: 5 minutes at 145°C (at 90 W power), 10 minutes at 190°C (at 90 W power), and a final 10 minutes at 75°C (at 90 W power). For digestion, 0.3 grams of dry plant samples were digested with 5 ml of HNO_3 and 2 ml of H_2O_2. The resulting solutions were cooled, brought up to a total volume of 25 ml with high-purity water, and then filtered through Whatman Grade 1 Qualitative Filter Paper with a pore size of 20-25 μm. The concentrations of cadmium, nickel, copper, lead, and zinc were measured using an ICP-OES (Model PerkinElmer, Optimal Emission Spectrometer 8000).

To calculate the concentration of heavy metals (C_{HM}) in plant tissues (stems and fruits) the formula (1) was used:

$$C_{HM}\ (\text{mg kg}^{-1}) = \frac{\text{AAS interpratation} \times \text{dillution factor}}{\text{dry weight of plant tissue}} \tag{1}$$

For the determination of the uptake capacity (UC) of the different plant tissues (g/ m^2) the formula (2) was used:

$$\textbf{UC (g/m}^{2}\textbf{) =} \frac{\text{Heavy Metal concentartion of plants tissue} \times \text{dry weight of plant tissue}}{10^{6}} \tag{2}$$

For the determination of the total uptake (TU) (g ha^{-1}), formula (3) was used:

$$\textbf{TU} = \text{Heavy metal uptake (Shoot)} + \text{Heavy metal uptake (seeds)} \tag{3}$$

4.4. Statistical Analysis

Statistical analysis was performed according to the experimental design using STATGRAPHICS Centurion XVII (version 1.0.1. C). As data followed normal distribution, One-way ANOVA was performed for comparing the growth and the phytoextraction ability of flax under the different nitrogen treatments among the two growing seasons. The LSD test was applied for the determination of the significant difference ($p<0.05$) between groups, at a confidence level of 95%.

5. Conclusions

In conclusion, our study reveals that nitrogen fertilization significantly affect flax productivity, with spring- sown flax showed better results to 30 kg N ha^{-1}, producing shorter but thicker plants and higher biomass (5.27 tn) compared to winter- sown flax (2.30 tn) treated with 60 kg N ha^{-1}. Flax shows a positive response to nitrogen fertilization and increased soil concentrations of heavy metals, making it a promising candidate for phytoremediation. The studied fiber flax variety can concentrate and uptake these metals in aboveground biomass following the order: Zn > Pb > Cd > Cu > Ni. Optimal fertilization levels vary based on soil characteristics, specific metals and cultivation seasons. Nitrogen application requires careful consideration because it is closely linked to growth promotion and biomass production. N1 treatment is more suitable for spring cultivation because it leads to increased heavy metal uptake of flax plants, whereas N2 nitrogen levels is more beneficial in winter cultivation. Climatic conditions significantly affect flax response to nitrogen rates and its phytoremediation ability. To sum up, our findings highlight the complex relationship between nitrogen fertilization, soil conditions and climatic factors in flax productivity and its potential for phytoremediation. These findings contribute to the development of targeted agricultural practices, which demonstrate how flax can be used for both biomass production and soil remediation.

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Appendix A

Climatic Conditions

The climatic data are derived from the METEOSEARCH data base [63]. These data are recorded by a weather station which is located at Lavreotiki peninsula. The Lavreotiki peninsula is characterized by its warm, dry summers and mild rainy winters which are typical of a Mediterranean climate. The weather conditions varied between the years 2022 (Figure 1) and 2023 (Figure 2). In the first growing period, the spring sowing season, the highest amount of rainfall was recorded in March 2022, reaching 25.0 mm, while the lowest was in May 2022, with only 1.8 mm of precipitation. In the second growing period, the winter sowing season, the peak in rainfall was recorded in January 2023, totaling 82.2 mm, and the minimum occurred in May 2023, reaching 5.8 mm of rainfall. The growing season of 2022 was characterized by relatively warmer climatic conditions compared to the second growing period in 2023, with higher temperatures recorded from May to July 2022. In the first cultivation period, the highest average temperature reached 27.8 °C in May 2022, while the lowest was 10.1 °C in March 2022. In contrast, during the second cultivation period, mean temperatures did not exceed 24 °C, particularly in June. During most cultivated months, and more specifically, from December to April, the mean temperature ranged between 10.8 °C and 15.8 °C, with May and June being the warmest months.

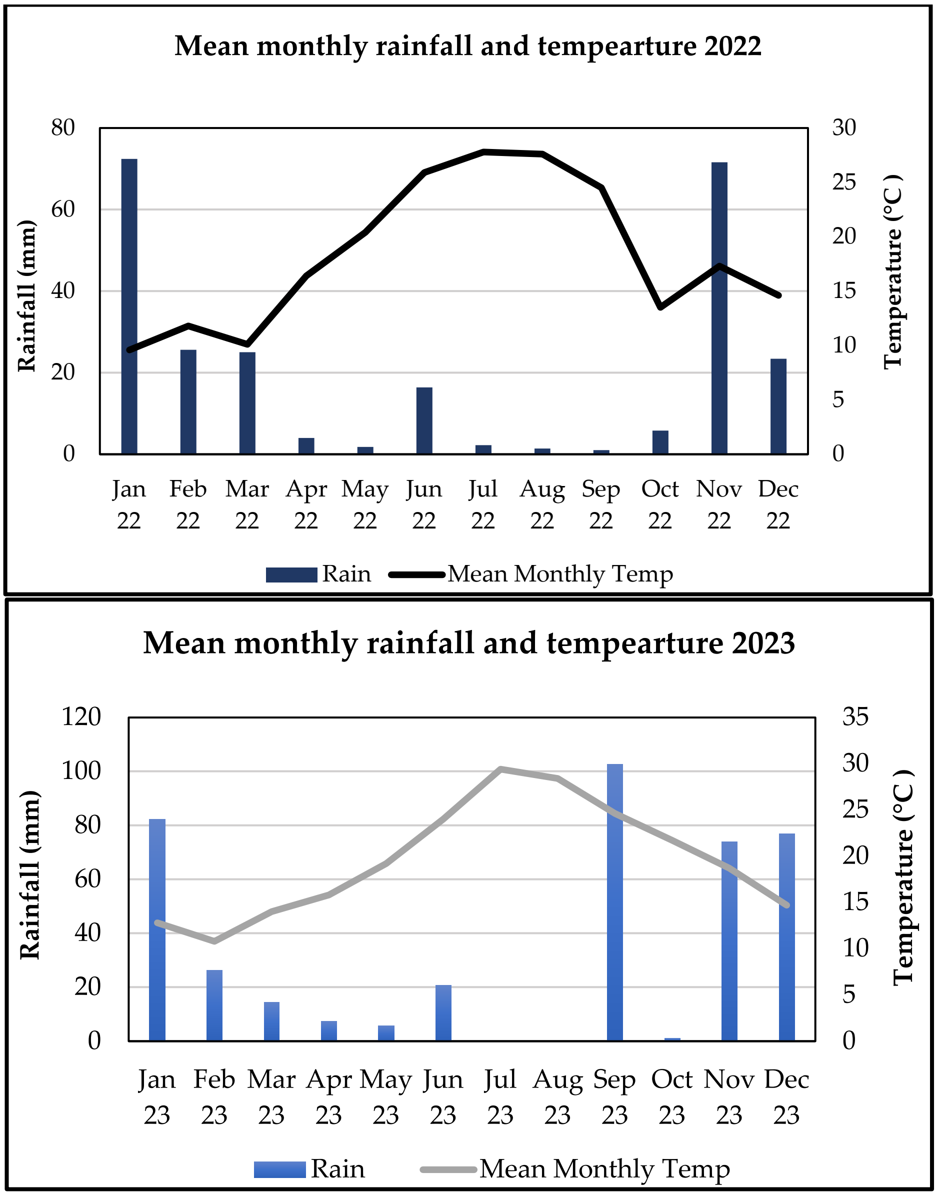

Figure 1. and 2. Mean monthly rainfalls and temperatures for the two experimental years (2022 and 2023) at the study site in Lavreotiki peninsula.

References

1. European Environment Agency. Available online: https://www.eea.europa.eu/data-and-maps/indicators/progress-in-management-of-contaminated-sites-3/assessment (accessed on 23.1.24).
2. Saleem, M.H.; Ali S.; Hussain, S.; Kamran, M.; Chattha, M.S.; Ahmad, S.; Aqeel, M.; Rizwan, M.; Aljarba, N.H.; Alkahtani, S.; Abdel- Daim, M.M. Flax (*Linum usitatissumum* L.): A Potential Candidate for Phytoremediation? Biological and Economical Points of View. *Plants* **2020**, *9*, 496. doi:10.3390/plants9040496.
3. Wuanna, R.A.; Okieimen, F.E. Heavy Metals in Contaminated Soils: A Review of Sources, Chemistry, Risks and Best Available Strategies for Remediation. *International Scholarly Research Notices* **2011**, *2011*, 402647. https://doi.org/10.5402/2011/402647.
4. Sharma, J.K.; Kumar, N.; Singh, N.P.; Santal, A.R. Phytoremediation technologies and their mechanism for removal of heavy metal from contaminated soil: An approach for a sustainable environment. *Frontiers in Plant Science* **2023,** *14*. https://doi.org/10.3389/fpls.2023.1076876.
5. Cleophas, F.N.; Zahari, N.Z.; Murugayah, P.; Rahim, S.A.; Yatim, A. N. M. Phytoremediation: A Novel Approach of bast Fiber Plants (Hemp, Kenaf, Jute and Flax) for Heavy Metals Decontamination in Soil-Review. *Toxics* **2022**, *11*. **https://doi.org/10.3390/toxics11010005.**

6. Zhao, X.; Guo, Y.; Papazoglou, E.G. Screening flax, kenaf and hemp varieties for phytoremediation of trace element- contaminated soils. *Industrial Crops & Products* **2022**, *185*, 115121. https://doi.org/10.1016/j.indcrop.2022.115121.
7. Heller, K.; Sheng, Q. C.; Guan, F.; Alexopoulou, E.; Hua, L.S.; Wu, G.W.; Jankauskiene, Z.; Fu, W.Y. A comparative study between Europe and China crop management of two types of flax: linseed and fiber flax. *Industrial crops and products* **2015**, *68*, 24-31. https://doi.org/10.1016/j.indcrop.2014.07.010.
8. Ceh, B.; Straus, S.; Hladnik, A.; Krusar, A. Impact of linseed variety, location and production year on seed yield, oil content and its composition. *Agronomy* **2020**, *10*. https://doi.org/10.3390/agronomy10111770.
9. Emam, S.M. Cultivars response of flax (*Linum usitatissimum* L.) to different nitrogen resources in dry environment. *Egypt Journal of Agronomy* **2019**, *41*, 119- 131. 10.21608/AGRO.2019.10947.1157.
10. Dordas, A.C. Variation of physiological determinants of yield in linseed in response to nitrogen feralization. *Industrial Crops and products* **2010**, *31*, 455- 465. https://doi.org/10.1016/j.indcrop.2010.01.008.
11. Angelova, V.; Ivanova, R.; Delibaltova, V.; Ivanov K. Bio-accumulation and distribution of heavy metals in fibre crops (flax, cotton and hemp). *Industrial crops and products* **2004**, *19*, 197-205. https://doi.org/10.1016/j.indcrop.2003.10.001.
12. Hosman, E.M.; El- Feky, S.S.; Elshahawy, M.I.; Shaker, M.E. Mechanism of phytoremediation potential of flax (*Linum usitissimum* L.) to Pb, Cd and Zn. *Asian journal of plant science and research* **2017**, *7*, 30-40.
13. Douchiche, O.; Chaibi, W.; Morvan, C. Cadmium tolerance and accumulation characteristics of mature flax, cv. Hermes: contribution of the basal stem compared to the root. *Journal of Hazardous Materials* **2012**, *101*, 235-236. http://dx.doi.org/10.1016/j.jhazmat.2012.07.027.
14. Bjelkova, M.; Gencurova, V.; Griga, M. Accumulation of cadmium by flax and linseed cultivars in field-simulated conditions: A potential for phytoremediation of Cd- contaminated soils. *Industrial crops and products* **2011**, *33*, 761-774. https://doi.org/10.1016/j.indcrop.2011.01.020.
15. Guo, Y.; Qiu, C.; Long, S.; Wang, H.; Wang, Y. Cadmium accumulation, translocation, and assessment of eighteen Linum usitatissimum L. cultivars growing in heavy metal contaminated soil. *International Journal of Phytoremediation* **2020,** 490-496. doi:10.1080/15226514.2019.1683714.
16. Zuk, M.; Richter, D.; Matuła, J.; Szopa, J. Linseed, the multipurpose plant. *Industrial Crops and Products* **2015**, *75*, 165-177. https://doi.org/10.1016/j.indcrop.2015.05.005.
17. Ma, H.; Guna, V.; Raju, T.; Narasimha Murthy, A.; Reddy, N. Converting flax processing waste into value added biocomposites. *Industrial Crops and Products* **2023**, *195*. https://doi.org/10.1016/j.indcrop.2023.116434.
18. Stavropoulos, P.; Mavroeidis, A.; Papadopoulos, G.; Roussis, I.; Bilalis, D.; Kakabouki, I. On the Path towards a "Greener" EU: A Mini Review on Flax (Linum usitatissimum L.) as a Case Study. *Plants* **2023**, *12*, 1102. https://doi.org/10.3390/plants12051102.
19. Kypritidou, Z.; Koourgia, P.M.; Argyraki, A.; Demetriades, A. Do humans take good care of their offsprig as animals do…! The Lavreotiki 'sagas', Hellenic Republic- Part1: historical outline and mapping of lead contamination. *Environmental Geochemistry Health* **2023**, *45*, 1107- 1116. https://doi.org/10.1007/s10653-021-01080-0.
20. Kalyvas, G.; Gasparatos, D.; Papassiopi, N.; Massa, I. Topsoil pollution as ecological footprint of historical mining activities in Greece. *Land degradation & Development* **2017**, *29*, 2025-2035.
21. Kontopoulos, A.; Komnitsas, K.; Xenidis, A.; Papassiopi, N. ENVIRONMENTAL CHARACTERISATION OF THE SULPHIDIC TAILINGS IN LAVRION. *Minerals Engineering* **1995**, *8*, 1209-1219. https://doi.org/10.1016/0892-6875(95)00085-5.
22. El- Bohramy, A.M.A.; Khedr, R.A.; El- Mansoury, M.A.M. Physiological, Biochemical and Agronomic response of some flax cultivars to water deficit under clay soil conditions in North Delta. *Journal of the advances in agricultural researches* **2022**, 351- 365, *27*. 10.21608/jalexu.2022.139346.1065.
23. Gabiana, C.; McKenzie, B.A.; Hill, G.D. The influence of plant population, nitrogen and irrigation on yield and yield components of linseed. *Agronomy Society of New Zeland* **2005**, *35*, 45-56. https://www.agronomysociety.org.nz/files/2005_6._Pop_N_irrigation_effects_on_linseed.pdf.
24. Goudenhooft, C.; Bourmaud, A.; Baley, C. Flax (*Linum usitissimum* L.) fibers for composite reinforcement: Exploring the link between plant growth, cell walls development, and fiber properties. *Frontiers in Plant science* **2019**, *10*. https://doi.org/10.3389/fpls.2019.00411.
25. Rossi, A.; Clemente, C.; Tavarini, S.; Angelini, L.G. Variety and sowing date affect seed yield and chemical composition if linseed grown under organic production system in a semiarid Mediterranean environment. *Agronomy* **2022**, *13*. https://doi.org/10.3390/agronomy13010045.
26. Soethe, G.; Feiden, A.; Bassegio, D.; Santos, R.F.; Melegari de Souza, S.N.; Secco, D. Sources and rates of nitrogen in the cultivation of flax. *African Journal of Agricultural Research* **2013**, *8*, 2249-2253. 10.5897/AJAR2013.7028.
27. Arslanoglu, S. F.; Sert, S.; Sahin, H.A.; Aytac, S.; El Sabagh, A. Yield and Yield Criteria of Flax Fiber (*Linum Usititassimum* L.) as Influenced by Different Plant Densities. *Sustainability* **2022**, *14*. https://doi.org/10.3390/su14084710.

28. Casa, R.; Russel, G.; Lo Cascio, B.; Rossini, F. Environmental effects on linseed (*Linum Usititassimum* L.) yield and growth of flax at different stand densities. *European journal of Agronomy* **1999**, *11*, 267-278. https://doi.org/10.1016/S1161-0301(99)00037-4.
29. Erdogdu, Y.; Yaver, S.; Onemli, F. The effect of different seeding rates on gain yield and yield components in some flax (*Linum usitatissimum* L.) varieties. *International journal of environmental & agriculture research* **2018**, *4*, 1-9.
30. Tavarini, S.; Castagna, A.; Conte, G.; Foschi, L.; Sanmartin, C.; Incrocci, L.; Ranieru, A.; Serra, A.; Angelini, L.G. Evaluation of chemical composition if two linseeds varieties as sources if health- beneficial substances. *Molecules* **2019**, *24*. 10.3390/molecules24203729.
31. El- Shimy, K.S.S.; Hammam, G.Y.M.; Allam, S.A.H.; Mostafa, S.H.A.; El- Gedwy, E.S.M.M. Flax yield potential affected by irrigation intervals and nitrogen fertilizer rates. *Annals of Agricultural Science* **2017**, *55*, 817- 824. DOI:10.21608/assjm.2017.57010.
32. Siedelka, A. Some aspects of interaction between heavy metals and plant mineral nutrients. *Acta societatis botanicorum Poloniae* **1995**, *64*, 265-272. https://doi.org/10.5586/asbp.1995.035.
33. Petrova, S.; Benesova, D.; Soudek, P.; Vanek, T. Enhancement of metal(loid)s phytoextraction by *Cannabis sativa* L. *Journal of food, agriculture and environment* **2012**, *10*, 631-641.
34. Shehata, S.M.; Badawy, R.K.; Aboulsoud, Y.I.E. Phytoremediation of some heavy metals in contaminated soil. *Bulletin of the National Research Center* **2019**, *43*. https://doi.org/10.1186/s42269-019-0214-7.
35. Kiran; Bharti, S.; Sharma, R. Effect of heavy metals: An overview. *Material Today: Proceedings* **2022**, *51*, 880-885.
36. Brutch, E.; Zabegaeva, O.; Nozkova, J.; Brutch, N. Cadmium tolerance and its absorption ability in fibre flax and linseed varieties. *Turkish Journal of Agriculture and Forestry* **2022**, *46*, 83-89. doi:10.3906/tar-2011-118.
37. Kakabouki, I.; Mavroeidis, A.; Tatridas, A.; Roussis, I.; Katsenios, N.; Efthimiadou, A.; Tiga, E.L.; Karydoyianni, S.; Zisi, C.; Folina, A.; Bilalis, D. Reintroducing flax (*Linum usitatissimum* L.) to the Mediterranean Basin: The importance of nitrogen fertilization. *Plants* **2021**, *10*. **https://doi.org/10.3390/plants10091758.**
38. Abdel- Kader, E.M.A.; Mousa, A.M.A. Effect of nitrogen fertilizer on some flax varieties under two different location conditions. *Journal of plant production* **2019**, *10*, 37-44.
39. Taddese, G.; Tenaye, S. Effect of nitrogen on flax (*Linum usitissimum* L.) fiber yield at debre behran area, Ethiopia. *Forestry research and engineering: International Journal* **2018**, *2*, 284- 286.
40. Istanbulluoglu, A.; Konukcu, F.; Kocaman, I.; Sener, M. The effect of deficit irrigation regimes in yield and growth components of linseed (*Linum usitissimum* L.). *Journal of Agricultural science and engineering* **2015**, *1*, 108-113.
41. Patel, R.K.; Tomar, G.S.; Dwivedi, S.K. Effect of nitrogen scheduling and nitrogen level on growth, yield and water productivity of linseed (*Linum usitissimum* L.) under Vertisols. *Journal Applied and natural science* **2017**, *9*, 698-705. https://doi.org/10.31018/jans.v9i2.1260.
42. Rahimi, M.M.; Zarei, M.A.; Arminian, A. Selection criteria if flax (*Linum usitissimum* L.) for seed yield, yield components and biochemical compositions under various planting dates and nitrogen. *African journal of agricultural research* **2011**, *6*, 3167-3175.
43. Zhang, Q.; Gao, Y.; Yan, B.; Cui, Z.; Wu, B.; Yang, K.; Ma, J. Perspective on oil flax and dry biomass with reduced nitrogen supply. *Oil crop science*, *5* **2020**, 42-46. https://doi.org/10.1016/j.ocsci.2020.04.004.
44. Pudelko, K.; Mankowski, J.; Kolodziej, J. Cultivation of fiber and oil flax (*Linum usitissimum* L.) in No- tillage and conventional systems. Part II. Influence of No- tillage and use of herbicides on yield and weed infestation of oil flax and the physical and biological properties of the soil. *Journal of natural fibers* **2015**, *12*, 72-83. 10.1080/15440478.2014.897669.
45. Chai, M.; Li, R.; Shen, X.; Tam, F.Y.N.; Zan, Q.; Li, R. Does ammonium nitrogen affect subcellular distribution and chemical factors of cadmium in *Kandelia obovata*? *Ecotoxicology and environmental safety* **2018**, *162*, 430-437. https://doi.org/10.1016/j.ecoenv.2018.07.031.
46. Grant, A.C.; Dribnenki, P. J.C.; Bailey, D.L. Cadmium and zinc concentrations and ratios in seed and tissue of solin (cv Linola ™ 947) and flax (cvs McGregor and Vimy) as affected by nitrogen and phosphorous fertilizer and Provide (*Panicillium bilaji). Journal of science of food and agriculture* **2000**, *80*, 1735-1743.
47. Tang, G.; Zhang, X.; Qi, L.; Wang, C.; Li, L.; Guo, J.; Dou, X.; Lu, M.; Huang, J. Nitrogen and phosphorus fertilizer increases the uptake of soil heavy metals pollutants by plant community. *Bulletin of environmental contamination and toxicology* **2022**, *109*, 1059- 1066. 10.1007/s00128-022-03628-x.
48. Hassan, M.U.; Chattha, M.U.; Khan, I.; Chattha, M.B.; Aamer, M.; Nawaz, M.; Ali, A.; Khan, M.A.U.; Khan, T.A. Nickel toxicity in plants: reasons, toxic effects, tolerance mechanisms, and remediation possibilities- a review. *Environmental science and pollution research* **2019**, *26*, 12673-12688. DOI:10.1007/s11356-019-04892-x.
49. Ahmad, M.; Ashraf, M. Essential roles and hazardous effects of nickel in plants. In: *Reviews of Environmental Contamination and Toxicology*, Whitcane D., Eds.; Springer: New York, 2011, pp. 125-167.
50. Chauhan, S.S.; Thakurand, R.; Sharma, G. NICKEL: ITS AVAILABILITY AND REACTIONS IN SOIL. Journal of Industrial Pollution Control **2008**, *24*, 1-8.

51. Shabbir, Z.; Sardar, A.; Shabbir, A.; Abbas, G.; Shamshad, S.; Khalid, S.; Natasha; Murtaza, G.; Dumat, C.; Shadid, M. Copper uptake, essentiality, toxicity, detoxification and risk assessment in soil- plant environment. *Chemosphere* **2020**, *259*, 127436. https://doi.org/10.1016/j.chemosphere.2020.127436.
52. Wei, B.; Yu, J.; Cao, Z.; Meng, M.; Yang, L.; Chen, Q. The Availability and Accumulation of Heavy Metals in Greenhouse Soils Associated with Intensive Fertilizer Application. *International Journal of Environmental Research and Public Health* **2020**, *17*, 5359. 10.3390/ijerph17155359.
53. Olivares, A.R.; Carrillo- Gonzalez, R.; Gonzalez- Chavez, M.C.A.; Hernadez, R.M.S. Potential of castor bean (*Ricinus communis* L.) for phytoremediation of mine tailings and oil production. *Journal of environmental management* **2013**, *114*, 316-323. https://doi.org/10.1016/j.jenvman.2012.10.023.
54. Shrestha, P.; Belliturk, K.; Gorres, H.J. Phytoremediation of heavy metal- contaminated soil by switchgrass: A comparative study utilizing different composts and coir fiber on pollution remediation, plant productivity and nutrient leaching. *International journal of environmental research and public health* **2019**, *16*. https://doi.org/10.3390/ijerph16071261.
55. Balafrej, H.; Bogusz, D.; Triqui, Z.-E.A.; Guedira, A.; Bendaou, N.; Smouni, A.; Fahr, M. Zinc hyperaccumulation in plants: a review. *Plants* **2020**, *9*, 562. 10.3390/plants9050562.
56. Broadley, M.R.; White, P.J.; Hammond, J.P.; Zelko, I.; Lux, A. Zinc in plants. *New phytologist* **2017**, *173*, 677-702. https://doi.org/10.1111/j.1469-8137.2007.01996.x.
57. Griga, M.; Bjelková, M.; Tejklová, *E.* Potential of flax (Linum usitatissimum L.) for heavy metal phytoextraction and industrial processing of contaminated biomass—a review. In Risk Assessment and Sustainable Land Management Using Plants in Trace Element-contaminated Soils COST Action 837. 4th WG2 Workshop, Bordeaux—Aquitaine, Villenave dˇıOrnon: France, 25th- 27th April, 2002.
58. Food and Agriculture organization of the United Nations. Available online: https://www.fao.org/publications/card/en/c/ed82a8fe-eab5-5880-bebb-5e22f342f55e/ (accessed on 8.12.2023).
59. Nelson, D.W.; Sommers, L.E. Total carbon, organic carbon, and organic matter. In *Methods of Soil Analysis, part 2 Chemical and Microbiological Properties*, 2nd ed.; Page, A. L., Miller, R.H.; Keeney, D.R., Eds.; American Society of Agronomy and the Soil Science Society of America: Madison, Wisconsin, USA, 1982; pp. 539-579.
60. U.S. EPA. *Method 3050B: Acid Digestion of Sediments, Sludges, and Soils*. Available online: https://www.epa.gov/esam/epa-method-3050b-acid-digestion-sediments-sludges-and-soils (accessed on 12.12.2023).
61. FAO. Available online: https://www.fao.org/3/cc0048en/cc0048en.pdf (accessed on 8.12.2023).
62. Flax Council of Canada. Available online: https://www.flaxcouncil.ca (accessed on 10.9.2023).
63. METEOSEARCH. Available online https://meteosearch.meteo.gr (accessed on 25.2.2024).