T.R.
ONDOKUZ MAYIS UNIVERSITY
INSTITUTE OF GRADUATE STUDIES
DEPARTMENT OF SOIL SCIENCE AND PLANT NUTRITION

EFFECTS OF ORGANIC AMENDMENT ON SOIL CHARACTERISTICS AND UPTAKE OF NICKEL BY ALYSSUM MURALE

Master's Thesis

Shreya THAPALIYA

Supervisor
Prof. Dr. Violina ANGELOVA

II. Supervisor
Prof. Dr. Rıdvan KIZILKAYA

SAMSUN
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SAMSUN
2022

.ACCEPTANCE AND APPROVAL OF THE THESIS

The study entitled **"EFFECTS OF ORGANIC AMENDMENT ON SOIL CHARACTERISTICS AND UPTAKE OF NICKEL BY ALYSSUM MURALE"** prepared by **Shreya THAPALIYA**, and supervised by **Prof. Dr. Violina ANGELOVA** and **Prof. Dr. Rıdvan KIZILKAYA**, was found successful and unanimously accepted by committee members as Master thesis, following the examination on the date 6.7.2022 .

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This thesis has been approved by the committee members that already stated above and determined by the Institute Executive Board.

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APPROVAL

… / … / …

Prof. Dr. Ali BOLAT

Head of Institute of Graduate Studies

DECLARATION OF COMPLIANCE WITH SCIENTIFIC ETHIC

I hereby declare and undertake that I complied with scientific ethics and academic rules in all stages of my Master's Thesis , that I have referred to each quotation that I use directly or indirectly in the study and that the works I have used consist of those shown in the sources, that it was written in accordance with the institute writing guide and that the situations stated in the article 3, section 9 of the Regulation for TÜBİTAK Research and Publication Ethics Board were not violated.

Is Ethics Committee Necessary?

Yes ☐ (If it necessary, please add appendices.)

No ☒

16/ 06/ 2022
Shreya THAPALIYA

DECLARATION OF THE THESIS STUDY ORIGINALITY REPORT

Thesis Title : EFFECTS OF ORGANIC AMENDMENT ON SOIL CHARACTERISTICS AND UPTAKE OF NICKEL BY ALYSSUM MURALE

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ÖZET

ORGANİK UYGULAMALARIN ALYSSUM MURALE TARAFINDAN NİKEL ALIMI VE TOPRAK ÖZELLİKLERİNE ETKİSİ

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Serpantinli topraklar, Doğu ve Orta Rodopların farklı yerlerinde geniş bir şekilde yayılış göstermektedir. Nispeten yüksek konsantrasyonlarda nikel ve diğer bazı metaller içeren serpantin topraklar, bazı bitkiler için, özellikle dokularında Ni biriktirenler için tercih edilen substrattır. Ni hiperakümülatörü Alyssum murale serpantin bölgesinde yaygın bir dağılım göstermekte ve biyokütlede %1-3'e kadar Ni biriktirebilmektedir. Bu çalışmanın amacı, denemede kullanılan serpantin toprağının temel toprak özelliklerini ve mineral bileşimini belirlemek ve organik madde ilavesinin toprak özellikleri üzerindeki etkisini ve nikel, mikro ve makro elementlerin alınabilir formlarının miktarını araştırmaktır. Bazı toprak ve bitki materyali örneklerinin mineralizasyonunda 10 MRD 300 rotorlu (10 konumlu) bir Milestone 1200 MEGA mikrodalga sistemi kullanılmıştır. Toprak ve bitki numunelerinin makro ve mikro element içeriklerini belirlemek için atomik absorpsiyon spektrometresi atomik emisyon spektrometresi ICP-AES kullanılmıştır. Yetiştiriciliğe organik katkı maddelerinin eklenmesinden sonra pH değerleri artmıştır. Çalışma sonunda toprak pH'sının metallerin hareketli formlarını etkilediğini belirlenmiştir. Alınabilir Ni formları ile pH ve DTPA ile ekstrakte edilebilir miktarları arasında önemli negatif korelasyonlar belirlenmiştir. Topraklara organik madde uygulanması, etkili olan dozla toprak KDK'sında önemli artış sağladığı saptanmıştır. Uygulanan kompost ve vermikompost miktarı %5'e yükseldikçe, KDK, kontrole (13.99 mmolc/kg) kıyasla sırasıyla 21.99 mmolc/kg ve 18.98 mmolc/kg'a yükseldiği belirlenmiştir. Organik materyal uygulanması toprağın alınabilir Ni (DTPA) içeriğinde azalmaya yol açdığı ve bu azalma tüm uygulamalarda %5 katkı uygulamasında daha belirgin olduğu bulunmuştur. En belirgin azalmanın ise %5 vermikompost (77,4 mg/kg) uygulamasında sağlandığı belirlenmiştir. Organik materyal uygulanması, bitki gelişimi üzerinde belirgin bir faydalı etkiye sahiptir ve bitkinin toprak üstü biyokütlesini artırdığı saptanmıştır. %5 aktif kömür ve %5 biochar ilave edilmiş toprakta bitki biyokütlesinin sırasıyla 665 mg ve 766 mg'a yükseldiği belirlenmiştir. Bununla birlikte, en önemli artışın kompost uygulamasında sağlandığı ve bitki biyokütlesinde 1281 mg (%2.5 kompost) ve 1852 mg (%5 kompost) artışa neden olduğu bulunmuştur. Organik materyallerin ürün verimi üzerinde olumlu bir etkiye sahip olduğu, %5 kompost uygulamasında, Ni veriminin kontrolden üç kat daha yüksek olduğu saptanmıştır. Elde edilen sonuçlar, organik materyallerin topraklara ilavesiyle toprak koşullarının iyileştiği ve topraktan farklı elementlerin alınmasına yardımcı olduğu belirlenmiştir.

Anahtar Sözcükler: Alyssum murale, Hiperakümülatör, Nikel, Fitoekstraksiyon, Serpantin

ABSTRACT

EFFECTS OF ORGANIC AMENDMENT ON SOIL CHARACTERISTICS AND UPTAKE OF NICKEL BY ALYSSUM MURALE

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Serpentine soils are widely distributed in different parts of the Eastern and Central Rhodopes. Serpentine soils, which contain relatively high concentrations of nickel and some other metals, are the preferred substrate for some plants, especially those that accumulate Ni in their tissues. The Ni hyperaccumulator Alyssum murale is widely present in serpentine region and can accumulate upto 1-3% Ni in biomass. The objective of this study was to determine the main soil characteristics and mineral composition of the serpentine soil used in the experiment and to investigate the influence of organic additives on soil characteristics and the amount of phytoavailable forms of nickel, micro and macro elements. A Milestone 1200 MEGA microwave system with a 10 MRD 300 rotor (10 positions) was used in the mineralisation of some of the soil and plant material samples. An atomic absorption spectrometer and atomic emission spectrometer ICP-AES were used to determine the macro-and trace element contents of soil and plant samples. After introducing organic additives in the cultivation, the pH values increased.The results of this study indicate that pH affects the mobile forms of metals. Significant negative correlations were found between pH and DTPA- available forms of Ni. The application of organic additives leads to an increase in the soil CEC, the dose being influential. As the amount of compost and vermicompost applied increased to 5%, the CEC increased to 21.99 mmolc/kg and 18.98 mmolc/kg, respectively, compared to the control (13.99 mmolc/kg). The application of organic additives leads to a decrease in the soil's bioavailable Ni (DTPA) content, and this decrease is more pronounced at 5% additive application in all treatments. The most significant decrease was observed with the application of 5% vermicompost (77.4 mg/kg). The application of organic additives had a pronounced beneficial effect on plant development and increased aboveground plant mass. When 5% activated charcoal and 5% biochar were added, the biomass increased to 665 mg and 766 mg, respectively. However, the most significant increase was with the application of compost, leading to an increase in plant biomass to 1281 mg (2.5% compost) and 1852 mg (5% compost). The results obtained after calculating the yield showed that the organic additives had a positive effect on the yield. In the variant with a 5% compost application, the Ni yield was three times higher than the control. Results obtained suggest that addition of organic amendments helps to improve the soil condition and uptake of different elements from the soil.

Keywords: Alyssum murale, Hyperaccumulator, Nickel, Phytoextraction, Serpentine soils

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LIST OF SYMBOLS AND ABBREVIATIONS

Ca	:Calcium
EC	:Electrical conductivity
K	:Potassium
Mg	:Magnesium
N	:Nitrogen
Ni	:Nickel
Na	:Sodium
P	:Phosphorus
pH	:Power of Hydrogen
Fe	:Iron
Zn	:Zinc
Mn	:Manganese
Si	:Silicon
Co	:Cobalt
Pb	:Lead
Cd	:Cadmium
ISO	:International Organisation for Standarization
DPTA	:Diethylenetriamine pentaacetate
TOC	:Total Organic Carbon
Db	:Bulk density
HCl	:Hydrochloric Acid
CEC	:Cation Exchange Capacity
WHC	:Water Holding Capacity
NaOH	:Sodium hydroxide
K_2SO_4	:Potassium sulphate
HNO_3	:Nitric Acid
$CaCO_3$	:Calcium carbonate
OM	:Organic Mater
AWC	:Available water content
PAW	:Plant available water
$BaCl_2$	:Barium Chloride

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1. INTRODUCTION

The largest serpentine bodies can be found in Bulgaria's Rila, Vlahina, Ograzhden, and Eastern and Central Rhodopes mountains. All over the Balkans, serpentine soils can be found. The region has an abundance of endemic species, both local and regional (Pavlova, 2010). Ultramafic (serpentine) soils have produced the most hyperaccumulators thus far, in part because of the inherent distinctiveness of the associated ultramafic flora (Anderson, et al., 1999). Some plants, especially those that store Ni in their tissues, appreciate the high nickel and other metal content of serpentine soils (Brooks and Radford, 1978). The Alyssum murale species are endemic to serpentine (derived from ultramafic) soils throughout Mediterranean Europe, but unlike many serpentine-endemic species, they thrive and grow rapidly away from their natural habitat (Reeves et al., 2000). Additionally, A. murale has the capacity to hyper-accumulate Ni in non-serpentine soils such as organic and limestone soils (Broadhurst et al., 2008).

It is believed that plants can thrive on serpentine soils due to a phenomenon known as hyperaccumulation. Hyperaccumulators are those that contain 10,000 mg of Zn or Mn per kg of dry weight. Along with metal tolerance, allelopathy, herbivore protection, and general disease resistance are all thought to be advantages of hyperaccumulation for the plant. There are at least 400 known Ni hyperaccumulators. In industrial and ultramafic environments, a Ni hyperaccumulator called alyssum murale can be found (Bani et al., 2007).

The Nickel hyper-accumulating plant Alyssum murale has attracted the most interest due to its remarkable capacity to extract and accumulate Ni from soils. Due to their capacity to remove sizable amounts of metals from soil, these hyperaccumulators are attracting the attention of researchers who are interested in phytoremediation (also known as phytoextraction), a practical, environmentally friendly method for decontaminating polluted areas (Abou-Shanab et al., 2003).

The discovery of hyper-accumulators of Ni has reignited interest in these species because of the unique issues that arise when a generally phytotoxic element is accumulated to such an incredible degree by plants. Research on phytochemistry has also been encouraged because it is now possible to isolate milligram quantities of metal complexes for further study, as opposed to the microgram levels envisaged with non-accumulating species (Homer et al., 1991). Nickel (Ni) phytomining is the process of growing specific Ni hyperaccumulator plants (also known as "metal crops") on low-value ore bodies, mineralized (ultramafic) soils, or anthropogenic metal-rich materials (such as contaminated soils, mine wastes, and industrial sludges), and processing the biomass after harvesting to recover valuable products like Ni metal or Ni salts (Nkrumah et al., 2019).

Manures, municipal biosolids, and other organic wastes can be used to supplement soil to improve its physical and chemical properties. The addition of organic soil can reduce soil bulk density and increase water holding capacity, aggregate stability, saturated hydraulic conductivity, water penetration rate, and biochemical activity, among other advantages (Zebarth et al., 2015).

The objective of the thesis is to carry out a comparative investigation to ascertain the impact of organic soil additives on the uptake of nickel by Alyssum murale and the potential applications of their use for phytoextraction of nickel from Bulgarian serpentine soils. It is important to do the following particular tasks in order to attain the goal as stated:

i. To identify the primary soil properties and mineral make-up of the serpentine soil that was ployed in the experiment.

ii. To research the effects of organic additions (compost, vermicompost made from Californian worm processing, activated carbon, and biochar) on soil properties and the quantity of phytoavailable forms of nickel, micro and macro elements.

iii. To compare the impact of particular organic amendments on the tested plant's accumulation of nickel and other micro and macro components.

iv. To assess the viability and efficiency of the considered soil additives for nickel phytoextraction from Bulgarian serpentine soils.

2. REVIEW OF LITERATURE

2.1. Nickel sources

The 24th most common element in the Earth's crust, nickel (Ni), makes up about 3% of the crust's total mass. It is the fifth most abundant element in terms of weight, behind silicon, magnesium, oxygen, and iron. It belongs to the transition series and is a member of group VIII B of the periodic table, which also contains iron, cobalt, platinum, palladium, and five other elements. Several different mineral formations include the naturally occurring metal nickel. As a transition metal, it is impervious to corrosion caused by air, water, and alkali, but it dissolves quickly in weak oxidizing acids. Nineteen unstable and five stable isotopes combine to form the natural isotopic composition of nickel. Numerous commercial and industrial uses use nickel and nickel compounds. Stainless steel and other nickel alloys with superior corrosion and temperature resistance make up the majority of nickel's uses. The metallurgical, chemical, and food processing industries use nickel metal and alloys extensively as pigments and catalysts. The most significant nickel salts from a commercial standpoint are oxide, sulphate, carbonate, hydroxide, nitrate, and chloride. One of the many trace metals that are widely dispersed in the environment and that come from both stationary and mobile sources is nickel. Both natural and man-made sources of nickel emit metal. It is present in biological matter, water, soil, and the atmosphere. Natural sources of atmospheric nickel include wind-borne dust, soil and rock weathering products, volcanic emissions, plants, and forest fires. Coal, diesel, and fuel oil burning, as well as the incineration of trash and sewage, among other sources, all result in the release of nickel into the atmosphere. Environmental sources of nickel include cheap jewelry, stainless steel kitchen utensils, dental or orthopedic implants, and tobacco. One more significant non-occupational nickel exposure factor is tobacco use. According to estimates, each cigarette will contain 1.1 to 3.1 g of nickel, with 10 to 20 percent of the nickel exhaled being in the gaseous state. According to some authors, cigarette smoke may contain nickel in the form of nickel carbonyl, which is especially dangerous to human health. Cigarettes, pipe tobacco, and other tobacco products have relatively similar nickel contents (Cempel and Nikel, 2005).

2.1.1. Nickel's natural sources

Nature is rich in nickel, which makes up about 0.008% of the earth's crust (Nickel, 2000). One of the many trace metals that are widely dispersed in the environment and that come from both stationary and mobile sources is nickel. Both natural and man-made sources of nickel emit metal. It is present in biological matter, water, soil, and the atmosphere. Natural sources of atmospheric nickel include wind-borne dust, soil and rock weathering products, volcanic emissions, plants, and forest fires. Although nickel is equally distributed throughout the soil profile, industrial and agricultural activity causes it to tend to accumulate at the surface. In areas close to cities, at industrial facilities, or even on agricultural land that gets sewage sludge, nickel contamination could be a significant problem. Between 3 and 1000 mg/kg can be found in soil. There are several different ways that nickel can be found in soils, including as inorganic crystalline crystals or precipitates, complexed or adsorbed on organic cation surfaces or inorganic cation exchange surfaces, and water-soluble, free-ion or chelated metal complexes in soil solution (Cempel and Nikel, 2005)

Igneous rocks are the primary source of most of the nickel in soils. The overall amount of a trace element, such as Ni, in soils is directly related to the parent material's concentration of the element as well as weathering processes. Numerous processes, including as leaching, runoff, degraded waste, and harvested goods, cause Ni to be lost from soil. Soil eroded from other areas and agricultural chemicals are two natural sources of nickel in soil (Chauhan et al., 2008).

2.1.2. Nickel availability

The highest values for nickel concentrations in ambient air are found in areas that are highly industrialized. Typically, airborne nickel concentrations range from 0.00001 to 0.003 g/m3 in remote locations, from 0.003 to 0.03 g/m3 in urban regions without metallurgical businesses, and from 0.07 to 0.77 g/m3 in nickel processing zones. In Poland, 0.025 g/m3 of nickel is the recommended atmospheric concentration. Industrial processes affect occupational nickel exposure, which is typically substantially higher than nickel exposure from outside of the workplace. The type of nickel to which workers are exposed varies by industry and occurs through dermal contact or inhalation (inhalation being the most prevalent route of exposure), with ingestion happening in cases where workplace cleanliness practices are weak. The most frequent causes include inhalation of dust containing relatively insoluble nickel compounds, aerosols made from nickel solutions (soluble nickel), and gaseous forms containing nickel. Workplace nickel concentrations have been shown to range from micrograms to milligrams per cubic meter of air in numerous high-risk workplaces (such as casting, welding, battery manufacture, etc.). The retention of 100 g of nickel per day occurs in industries that produce or use nickel when about 0.2% of the workforce is exposed to high amounts of airborne nickel (Uren, 1992).

Typically, drinking water contains less than 10 g/l of nickel. Assuming a daily intake of 1.5 liters of water and a level of 5–10 gNi/l, the mean daily intake of nickel for people via water would range from 7.5 to 15.0 g. In the United States, 2053 drinking water samples were examined, and 97 percent of the samples had nickel contents that were less than 20 g/l or less than 10 g/l. Values as low as 75 g/l have been reported in sporadic situations, whereas values as high as 200 g/l have only been discovered in nickel ore mining areas. It is exceedingly unlikely that rising nickel levels in drinking water would ever cause health issues. The average Ni level of 80 drinking water samples taken in Poland from a region impacted by industrial pollution was 17 g/l, with the majority of the samples tested below the allowed limit of 20 g/l. The caliber of the pipes affects the amount of Ni in both cold and hot water. When using metal pipes, the level of nickel is lower in hot water than it is in cold water. The concentrations are inverted when PVC pipes are used, though. By using soft water and acidic beverages, nickel can be removed from pipes and containers. By means of leaching or corrosion, oral nickel intake can rise significantly, up to 1 mg per day in some instances. The morning flushing reduced nickel levels in drinking water in screened households considerably, going from 10.79 g/l to 7.23 g/l, respectively (Kierczak et al., 2021)

The largest contributors to trace metal contamination in aquatic ecosystems, including the ocean, are non-ferrous metal smelters and domestic wastewater effluents, particularly those containing As, Cr, Cu, Mn, and Ni (Cd, Ni, Pb and Se). Since phytoplankton and other aquatic plants are sensitive bio-indicators of water pollution, nickel accumulates quickly in the biota, particularly in these organisms. Its deposition in the sediment may be aided by precipitation, complexation, adsorption on clay particles, uptake by biota, and other processes. Lakes are characterized by the ionic form and its interactions with organic substances. Based on in-depth analyses of lakes, it was discovered that there is heavy metal and acid oxide precipitation in the Lake Kochejavr catchment region (more than 100 km away from the nearest source of pollution - copper nickel industry companies). Biological processes in fresh water catchments were found to be dangerous at Ni precipitation levels of 0.9 mg/m2 /year over extended periods of time. In rivers, nickel is primarily spread as a precipitated coating on particles and in combination with organic components. The River Karoon, which is the only navigable river in southern Iran, has nickel concentrations that varied from 41.0 to 60.7 g/l in the spring to 69.3 to 110.7 g/l in the winter. The results show that pollution along the river has expanded all the way to the estuary of the Persian Gulf. Rivers and streams also discharge some nickel into the ocean. Rivers in Poland are frequently used to transfer nickel into the Baltic Sea; the average anthropogenic Ni inflow is 57 percent. Nickel concentrations in seawater range from 0.1 to 0.5 g/l (Chauhan et al., 2008).

Although nickel is equally distributed throughout the soil profile, industrial and agricultural activity causes it to tend to accumulate at the surface. In areas close to cities, at industrial facilities, or even on agricultural land that gets sewage sludge, nickel contamination could be a significant problem. Between 3 and 1000 mg/kg can be found in soil. Nickel can be found in soils in a number of different ways, such as inorganic crystalline solids or precipitates, complexed or adsorbed on organic cation surfaces or inorganic cation exchange surfaces, and water soluble free-ion or chelated metal complexes in soil solution. The mobilization of this metal as a result of increasing acid rain and decreased soil pH due to diminished soil liming in agriculture does not now appear to be a major concern outside of urban areas, but it might be in the future. Mielke et al. looked at the effect of anthropogenic metals on the geochemical quality of urban soils. Urban alluvial soils exhibited a median nickel value of 9.8 g/g, whereas fresh alluvium samples had a median nickel level of 3.9 g/g (New Orleans and stratified by census tracts). Overall, the metropolis has far greater metal values than the outskirts, which are significantly lower. The average concentration of nickel was higher (average 17.20 mg/kg) in 60 soil samples from the Polish region of Stalowa Wola, which is affected by industrial pollution, than in the reference samples (average 9.72 mg/kg). All of the readings, though, fell below the allowable maximum concentration. The maximum allowable concentration of nickel in soils in allotment gardens in post-flooded industrialized districts was not exceeded in the Dolnolski Region in 2000–2001. Current Polish legislation state that the permissible limit for nickel in soil is 50 mg/kg d.w. in non-industrialized areas and is based on a variety of factors (Wang et al., 2020).

When used as a primary element in food for humans, nickel is regarded as a common part of the diet, and its derivatives are typically regarded as safe. There is little information available about the chemical forms of nickel present in various diets or whether dietary nickel can be found in unique "organic" forms with higher bioavailability, akin to iron and chromium. Meals can have nickel values of less than 0.1 mg/kg to 0.5 mg/kg. Some foods may have absorbed nickel during manufacture, but the majority seem to have done so naturally. By milling wheat, catalytic hydrogenation of lipids and oils using nickel catalysts, and leaching from nickel-containing alloys in stainless steel food processing equipment, food processing techniques appear to raise nickel levels in foods. Due to their high nickel content, foods like oatmeal, dry beans and peas, almonds, dark chocolate, and soy products can cause people to consume up to 900 g of nickel per day. There isn't enough evidence to support a nickel requirement in humans yet. Research shows that the amount of nickel consumed through food varies greatly per country, but the average daily intake in the majority of them is between 100 and 300 grams. According to 66 liters of wine per resident per year, the average French person consumed 30.6 g of nickel every week (4.37 g per day), according to estimates. Nickel levels in a variety of food products were evaluated in Poland. 1990 saw a survey of 27 full-day rations at canteens in Lublin and Warsaw as well as the food rations of families of manual laborers in many Polish municipalities. Current dietary guidelines state that daily nickel intake levels of 187–302 g for canteen rations and 183–341 g for family rations are considered safe. According to Leszczyska and Gambu, 132 g/day of nickel was consumed by adult Polish residents of specific farms located inside an industrial region between 1996 and 1998. Various fruits and vegetables, fruit and vegetable juices, wine, and cocktails all have safe levels of nickel. Similarily, nickel concentrations in several natural ground coffees, instant coffee brands, coffee beans, and coffee infusions did not exceed the legal limits for concentrations permitted in Poland. On the other hand, there was a significant association between the amounts of nickel in coffee beans and coffee infusions. Bee honey, candies, dried herbs, tea leaves, and granulated tea all contained less nickel than permitted levels (Kierczak et al., 2021)

2.2 Serpentine soil

Serpentine soils are weathered ferromagnesian silicate-rich ultramafic rocks. Serpentine soils are often low in important plant nutrients including nitrogen, phosphorus, potassium, and sulphur, as well as heavy metals like nickel, cobalt, and chromium. Although the physical characteristics of serpentine soils vary greatly from site to site and within a site, serpentine soils are frequently found in open, steep landscapes with shallow, rocky substrates and little moisture retention capacity. Plants that grow in serpentine soils give genetic material for phytoremediation and phytomining. Rocks containing the serpentine group of minerals (hence, serpentine rocks or serpentinite) can be found on practically every continent and island arc, and they originate in the upper mantle of the Earth. The word serpentine comes from the Latin serpentinus, which means "serpent," and refers to the olive-green hue and smooth, scaly, or mottled look of many serpentine-containing rocks. Serpentine soils are difficult settings for plants, and there has been a lot of interest in Figureuring out how they deal with them. Plant growth is hampered by serpentine soils' low Ca:Mg ratio, and strategies to cope with this low ratio are key adaptations. High levels of soil Mg tolerance, as well as reduced Mg absorption or increased Ca absorption, are examples of these mechanisms. Palm et al. (2012), for example, use the model plant Mimulus guttatus to demonstrate that Mg exclusion does not explain serpentine plants' capacity to flourish in hydroponic solutions with low Ca:Mg ratios. Low macronutrient concentrations, excessive amounts of some heavy metals, and a lack of water have all been mentioned as contributing reasons. Serpentine environments may be hazardous to human health. First, water from serpentine watersheds may have elevated metal content. High Ni levels were found in streams draining from a serpentine watershed in central Europe, according to Krám et al. Even if serpentine soils are adjusted to allow agricultural growth, excessive soil metal concentrations may lead to high amounts in crops, posing a health risk to humans (Boyd and Rajakaruna, 2014).

2.2.1 Serpentine rocks and soils chemical properties

Serpentine rock is a magnesium iron silicate that forms when peridotite metamorphoses. Although peridotite is mostly olivine, it also contains various amounts of pyroxenes and amphiboles, thus some Ca, Al, and Na are present. In several cases, chromite, $FeCr_2O_4$, and garnierite are present in significant amounts. The color of the rock reflects these differences in composition, which can range from gleaming green to blue or almost black. At the surface, rock-formed soils are typically reddish, brown, or grey, turning to yellowish or greenish in the lower layers. Because the physical and chemical features of soils made from unserpented peridotite and peridotite serpentine mixtures are similar, it will be easier to refer to all of these related forms as "serpentine soils" in this article. Because the underlying rocks have such a wide variety of compositions, the compositions of the derived soils should also vary significantly, particularly in secondary elements like calcium, potassium, and heavy metals. However, even minor variations in these elements can have a significant impact on the soil's ability to support plant growth. While the entire composition of the parent rocks is only one of the components involved in the production of soil, examinations of some of these fundamental igneous rocks, as well as soils of this origin, are of major interest. The principal constituents, Si, Mg, and Fe, are all in the same range in the rocks; aluminium is absent in all cases; and all other elements have values under 1%. Although the amounts of calcium and heavy metals in serpentine soils are tiny, they will be seen to play a crucial part in some of the theories that attempt to explain plant intolerance. Weathering reduces magnesium content, which is substantially lower in soils than in rocks; in most situations, magnesium is lower in the surface soil than in deeper soil layers, according to Robinson et al. (1935). The loss of this element is particularly obvious in tropical soils like that of New Caledonia, where iron build-up is also noticeable. Chromium is also particularly abundant in tropical soils, either as a result of its richness in the parent rock or as a result of a build-up comparable to that of iron. It's debatable if the above total analyses provide realistic estimates of the amounts of various ions accessible for plant absorption. The determination of exchangeable cations using the ammonium acetate extraction method is one of the most important types of tests used to evaluate the quantities of ions available to plants. Except in New Caledonia's tropical soil, magnesium far outnumbers calcium in the table. The findings also reveal that, despite the fact that all serpentine soils are low in calcium, there is a wide variation of calcium levels. It will be seen later how such variances might have a significant impact on a soil's sterility (Rajakaruna & Boyd, 2014).

2.2.2 Serpentine soil physical characteristics

Peridotite and serpentine formations are common intrusions of vast depth and often of old geologic origin; consequently, it is reasonable to expect deep soils, as is the case in many of Maryland's Conowingo soils and in tropical soils discussed above (Robinson et al., 1935). Many serpentine sites, on the other hand, are in rolling or mountainous terrain, and the soils there are typically shallow, owing to the steepness of the slopes and the sparseness of the flora, which encourages erosion. In many cases, these soils are mostly composed of relatively unweathered rock particles and are referred to as lithosols; in others, the shallow soils contain significant clay and organic content and are referred to as azonal soils of a generally stable character. Regardless of topography, Robinson et al. (1935) feel that some serpentine rocks are too deficient in alumina to create adequate clay for the development of a regular soil covering. This concept about insufficient clay production could explain why only a thin soil mantle exists on mountainous rather than level places. The soil textures range from heavy clays to light loams, with the most frequent kind in the western United States being a friable, gravelly loam. Robinson et al. (1935) found through a series of extensive physical analyses that serpentine soils have no physical characteristics that would make them particularly unfavorable for plant growth, however the clay concentration of the subsoils in certain cases was high enough to obstruct drainage. Undesirable physical qualities, such as soil mantle shallowness, gravelly texture, low clay content, and steepness of topography that fosters erosion, have a significant impact on serpentine vegetation because they result in lower moisture and nutrient levels. Plant cover is more numerous on moister serpentine areas, such as north slopes, and often includes extra species that are absent in drier circumstances. This suggests that plants that are common on serpentine soils have drought resistance as well as tolerance for serpentine soils, at least in the western United States. However, because different physical parameters may have different effects in soils of different parent materials, the idiosyncrasies of serpentine soils do not appear to be explainable by physical properties (Boyd and Rajakaruna, 2014).

2.2.3 Nickel, chromium and other heavy metal toxicity in serpentine soil

It has been proposed that toxic concentrations of heavy metals such as chromium and nickel are to blame for serpentine soils' unfavorable plant growth conditions (Robinson et al., 1935). Unquestionably, serpentine soils have higher chromium and nickel content than soils of other origins, and plants can absorb significant amounts of these metals. In the dry leaf material of Pancheria glabrosa growing on a tropical soil with a pH of 5.8, the amounts absorbed could be as high as 86 parts per million Ni and 4.5 parts per million Cr. These values are significantly higher than those found in tissue grown in the field or in a greenhouse from several neutral serpentine soils in the western United States. Little is known about the parameters that influence nickel and chromium absorption from soils, however it is likely that pH, the type of primary and secondary minerals, anions such as phosphate concentrations, and maybe other soil features have a significant impact on the heavy metals' uptake. A glacial area in Scotland where the parent material is mostly serpentine rock, emphasized the importance of pH in nickel availability. Oats grown on these soils accumulated 16 to 134 parts per million Ni in their expanded leaves, with the more acid soils absorbing the largest amounts and the less acid fields absorbing the smallest amounts. In the leaves of immature plants, concentrations of 30-40 parts per million showed moderate toxicity symptoms, while higher concentrations caused more severe chlorosis and necrosis, which appeared in longitudinal stripes. The writer observed toxicity symptoms in tomato and sunflower (Helianthus) plants cultivated in culture solutions when the concentrations of chromium or nickel in the solutions were high enough to significantly decrease plant growth. Tomato and sunflower were produced with great development and no toxicity symptoms on numerous serpentine soils from the western United States (in which the calcium was elevated to a suitable level). Of again, this could just be more proof that plants take less nickel from these almost neutral soils than from more acidic soils. Another facet of toxicity that has to be investigated in serpentine soils is the possibility that adding molybdenum to culture solutions could ameliorate toxicities produced by excessive nickel, manganese, or other cations. Some acid serpentine soils can absorb large levels of chromium and nickel, whereas neutral soils have much less available to plants. While this toxicity factor is significant in some areas, it appears unlikely that it is the solecause of the serpentine habitat's global uniqueness (Whittaker, 1954).

2.3 Metal hyper-accumulator plants

A group of plants known as "hyperaccumulators" are from many families and have the capacity to grow on metalliferous soils and accumulate extraordinarily high amounts of heavy metals in their aerial organs—far above those seen in most species—without experiencing phytotoxic effects. Three key traits set hyperaccumulators apart from comparable non-hyperaccumulating taxa: a markedly higher rate of heavy metal uptake; a quicker root-to-shoot translocation; and a superior capacity to detoxify and sequester heavy metals in leaves. Among the theories put out to explain the purpose of hyperaccumulation, the "elemental defense" hypothesis, which contends that plants hyperaccumulate heavy metals as a defense mechanism against natural enemies like herbivores, has garnered the greatest support. According to the more modern theory of "joint actions," heavy metals can cooperate with organic defensive chemicals to enhance total plant resistance. The health of people and animals is increasingly threatened by heavy metal-contaminated soil. Over the past 20 years, remediation efforts have increasingly used plants that hyperaccumulate particular metals. For phytoremediation (the removal of pollutants from soils) or phytomining (the cultivation of plants to extract metals), metal-accumulating species can be used (growing plants to harvest the metals). Additionally, dietary fortification and phytoremediation may be viewed as complementing strategies because many of the metals that may induce hyperaccumulation are also essential nutrients (Rascio and Navari-Izzo, 2011).

Phytoextraction has been used to remove heavy metals, pesticides and xenobiotics, organic compounds, toxic aromatic pollutants, radionuclides, and acid mine drainage. A plant's reliance on the absorption of zinc, manganese, nickel, and copper to maintain natural function explains its capacity to phytoextract certain metals. The use of plants' inherent agronomic traits and benefits, such as high biomass, extensive root systems, and ability to withstand environmental stress, is the biggest benefit of using plants for environmental cleanup. Animals living in the impacted area may eat plants that absorb dangerous substances, contaminating the food chain. The term "hyperaccumulation" was created by Jaffre et al. in 1976 when they discovered Ni accumulation in Sebertia accumulata. Metal-hyperaccumulation is defined as the uptake and sequestration of extraordinarily high concentrations of heavy metal in the aboveground parts of a plant under field settings. A plant needs to be able to accumulate 0.1 percent of the dry mass of elements like Ni, Co, or Pb, 1 percent of the dry mass of Zn, Mn, and 0.01 percent of the dry mass of Cd to be categorized as a hyperaccumulator. These plants could be used to clean up contaminated soil because they are resistant to particular metal ions. Using plants to remove, extract, or inactivate metal ions from soil is a scientific and environmentally benign technique known as phytoremediation, which has been made possible by the use of hyperaccumulators (Shah and Nongkynrih, 2007).

There are known to be 400 Ni hyperaccumulators. In industrial and ultramafic environments, you can find a Ni hyperaccumulator called alyssum murale (Bani, et al., 2007). The Ni-hyperaccumulating plant Alyssum murale has drawn the most interest due to its remarkable capacity to extract and accumulate Ni from soils. Due to their capacity to remove sizable amounts of metals from soil, these hyperaccumulators are attracting interest from scientists interested in phytoremediation (also known as phytoextraction), a practical, environmentally friendly method for decontaminating polluted areas (Abou-Shanab et al., 2003).

The discovery of hyperaccumulators of Ni has aroused renewed interest in these species because of the unique issues that arise when a generally phytotoxic element is accumulated to such an incredible degree by plants. The ability to extract milligram quantities of metal complexes for further study has also spurred phytochemical research, as opposed to the microgram quantities envisaged with non-accumulating species (Homer et al., 1991). Nickel (Ni) phytomining is the process of growing specific Ni hyperaccumulator plants ('metal crops') on low-value ore bodies, mineralized (ultramafic) soils, or anthropogenic metal-rich materials (like contaminated soils, mine wastes, and industrial sludges), and processing the biomass after harvesting to recover valuable products like Ni metal or Ni salts (Nkrumah et al., 2019).

"Hyperaccumulators" are plants that actively take up exceptionally high quantities of one or more heavy metals from the soil, in contrast to excluder plants. Furthermore, heavy metal concentrations in hyperaccumulating species are 100–1000 times higher than in non-hyperaccumulating species because they are not maintained in the roots but rather translocated to the shoot and deposited in aboveground organs, notably leaves. They don't exhibit any phytotoxic symptoms. Hypertolerance, a crucial characteristic that enables plants to withstand heavy metal poisoning, to which hyperaccumulator plants are equally vulnerable as non-hyperaccumulator plants, is necessary for hyperaccumulation. Less than 0.2 percent of all known species, or roughly 450 angiosperm species, have been identified to possess heavy metal (As, Cd, Co, Cu, Mn, Ni, Pb, Sb, Se, Tl, Zn) hyperaccumulators. The fact that these plants are still being reported as occurring suggests that many hyperaccumulators have not yet been found in nature. On the other hand, if this trait is not supported by controlled testing, several species that were listed as hyperaccumulators based on field samples may be removed from the list. For instance, a full re-examination of the Cu/Co hyperaccumulators was triggered by the observation that Cu and Co hyperaccumulation by field samples in a number of cuprophytes was really caused by leaf surface contamination. The presence of the hyperaccumulator species in a wide variety of distantly related families suggests that the trait has independently developed more than once in response to certain ecological conditions. Hyperaccumulating plants are thought to have evolved for unknown and debatable reasons. However, a variety of options have been put out. They are covered in more detail in the section that follows. On metal-rich soils, heavy metal hyperacumulators can be found in both tropical and temperate regions. Latin American, North American, European, South African, and New Caledonian vegetations all contain them. The term "hyperaccumulator" was initially used to describe plants that could accumulate more than 1 mg g1 Ni (dry weight) in the shoot, which is a very high heavy metal concentration given that the toxicity of nickel in most plant vegetative organs is between 10 and 15 mg g1. Based on each heavy metal's distinct phytotoxicity, threshold values were offered in order to detect hyperaccumulation. When cultivated on native soils, hyperaccumulators accumulate more than 10 mg g1 (1 percent) of Mn or Zn, more than 1 mg g1 (0.1 percent) of As, Co, Cr, Cu, Ni, Pb, Sb, Se, or Tl, and more than 0.1 mg g1 (0.01 percent) of Cd in the aerial organs without experiencing phytotoxic effects. More than 75% of taxa hyperaccumulate Ni, while Cd, one of the most toxic heavy metals, has a much smaller proportion of taxa that do so (just 5 species to date). The metal that has been shown to accumulate in plants at the highest amounts is nickel. Sebertia acuminata (Sapotaceae), a tree specific to the serpentine soil of New Caledonia, demonstrates this by accumulating up to 26% Ni (dry mass) in its latex. The Brassicaceae family, notably the genera Thlaspi and Alyssum, contains about 25% of the recognized hyperaccumulators. The majority of Ni hyperaccumulating taxa also belong to these taxa. Brassicaceae plants such as Sedum alfredii, Arabidopsis halleri, and Thlaspi are Zn hyperaccumulators (Crassulaceae). The four identified species that hyperaccumulate Cd in addition to Zn are Thlaspi caerulescens, Thlaspi praecox, A. halleri, and S. alfredii. The sixth Cd hyperaccumulator has just been identified as Solanum nigrum (Solanaceae). Among the families having species that produce excessive amounts of Se include Fabaceae, Asteraceae, Rubiaceae, Brassicaceae, Scrophulariaceae, and Chenopodiaceae. A number of brake ferns from the genus Pteris have also been proven to hyperaccumulate, in addition to angiosperms from the Brassicaceae family including Isatis cappadocica and Hesperis persica. While other "facultative metallophytes" can live in non-metalliferous soils but are more prevalent in metal-rich environments, most hyperaccumulators are "strict metallophytes," meaning they exclusively exist in metalliferous soils. Additionally, populations of some species are both metallicolous and non-metallicolous. Some of them, like the Zn hyperaccumulators A. halleri and T. caerulescens, exhibit hyperaccumulation at the species level and in all populations. This characteristic is only present in metallicolous populations and is not constitutive at the species level in some, such the Zn hyperaccumulator S. alfredii and Cd hyperaccumulators (Shrivastava et al., 2019).

Between species, as well as between populations and ecotypes within the same species, the degree of hyperaccumulation of one or more heavy metals can vary significantly. On the other hand, hyperaccumulation is based on three distinctive traits that set hyperaccumulator taxa apart from non-hyperaccumulator taxa. These traits include a noticeably increased capacity for heavy metal absorption from the soil, a quicker and more efficient metal translocation from root to shoot, and a greatly increased capacity for heavy metal detoxification and sequestration in the leaves. In the past ten years, great progress has been made in understanding the mechanisms behind metal hyperaccumulation by comparative physiological, genetic, and proteomic studies of hyperaccumulators and equivalent non-hyperaccumulator plants. Numerous studies have focused on T. caerulescens and A. halleri, which have evolved into model plants. An unusual trait highlighted by this study is the fact that the majority of critical hyperaccumulation steps depend on genes shared by hyperaccumulators and non-hyperaccumulators, which are produced and controlled differently in the two types of plants (Memon et al., 2015).

Biologists were challenged to propose answers for this peculiar behavior by responding to the question: why do certain plants do it? after learning about a class of plants that concentrate extraordinarily high levels of typically harmful heavy metals in their leaves. In other words, what functions do these plants' metal hyperaccumulation processes serve, and what are the advantages and adaptive values of metal hyperaccumulation? Some of the theories put up to explain the function of high elements concentrations in leaves include metal tolerance/disposal, drought resistance, interference with nearby plants, and defense against natural predators. The peculiar hyperaccumulation pattern would enable plants to store heavy metals in tolerant leaf tissues, allowing them to be removed from the roots, in accordance with the tolerance/disposal concept. They are expelled from the plant body by shedding the high-metal aerial organ. Another hypothesis is that high concentrations of heavy metals increase plant tolerance to drought by functioning as osmolytes inside cells or as water-saving agents in cell walls. However, as there is little experimental data to support these hypotheses, further research into their validity is necessary. The interference theory, also referred to as "elemental allelopathy," contends that perennial hyperaccumulator plants may affect adjacent plants by enriching metal in the topsoil beneath their canopies. A high-metal leaf litter is produced as a result, which inhibits the growth of species that are less metal-tolerant. One group discovered higher Ni levels in the surface soil under the canopy of the hyperaccumulator S. acuminata than under the canopy of non-hyperaccumulator species. Alyssum murale's high-Ni leaves, according to another group, don't create a "poison zone" around the Ni hyperaccumulator or stop the germination of rival plants' seeds, contradicting the theory of elemental allelopathy. The absence of allelopathic effect is most likely caused by the fact that the majority of Ni released from leaf biomass quickly binds to soil components, preventing it from having an impact on neighboring plants. Additionally, there is little experimental evidence to support the elemental allelopathy concept (Rascio and Navari-Izzo, 2011).

2.4 Alyssum murale botany and characteristics

A member of the mustard family called yellowtuft, also known as Alyssum murale, does best in well-drained, rocky soils. The leaves are covered in tiny white hairs, giving the plant a grayish green appearance. Clusters of yellow blooms grow at the top of thin stalks that are between 10 and 24 inches tall. Only around 14 inches are across each flower. They have four petals and six stamens. The flat-topped flower clusters can reach a width of several inches. Particularly in rock gardens, horticultural species are sought for their decorative appeal. A. murale is also known as a "hyperaccumulator" plant, which means it can absorb metals like nickel and store them in its leaves, blossoms, and stems, allowing it to grow in potentially poisonous soils. It is capable of storing 30 g kg1 Ni in dry leaf biomass. In a process known as bio-remediation, researchers are interested in employing plants like A. murale as a natural method of cleaning up mining and other heavy metal sites. The Rhodope region was selected as a study area for Alyssum bioremediation because serpentine soils are usually high in nickel. In the spring, keep an eye out for the grayish green leaves; by late May, the bright yellow blossoms will be visible. Most likely, the plant would flourish in a rocky, serpentine setting (The Nature Conservancy, 2011).

2.5 Bioavailability of nickel

There are many metal contaminants in soils, sediments, and water, and they can be dangerous for both people and the environment. With an average concentration of 50 mg kg-1 and a concentration of 10,000 mg kg-1 in serpentine and peridorite bed rocks, nickel is the 24th most common element in the crust of the planet. Because of human activity, nickel pollution is already commonplace. The use of sewage sludge or biosolids, irrigation with sewage water, and releases of nickel from the steel and alloy industry, electroplating, nickel-cadmium batteries, electronic components, catalyst for fat hydrogenation, methanation, and petroleum products are the main causes of nickel contamination. Mobility and bioavailability of Ni play a major role in determining its toxicity. Bioavailability is the measure of a chemical's availability to a living biota. The qualities of the contaminant as well as the soil environment play a role in determining the amount of bioavailable contaminant in soil. Numerous soil characteristics, including pH, ion exchange capacity, organic matter, soil type, and time dependence, among others, have a significant impact on the availability and extractability of Ni in soils (Sathya and Mahimairaja, 2015).

A group of metal elements known as heavy metals can be dangerous to living things if they enter their systems. Contaminated aquatic habitats may contain mercury (Hg), chromium (Cr), nickel (Ni), zinc (Zn), cadmium (Cd), and lead (Pb). Because they seem to bioaccumulate, heavy metals are dangerous. As contrast to the concentration of a chemical in the atmosphere, bioaccumulation refers to a rise in the concentration of a chemical in a biological organism over time. Nickel is used in a wide range of products, including steel armor (stainless steel), copper shielding, batteries, electronics, aircraft industrial applications, textiles, gas-fired power generation turbines, strong magnet makers, nichrome laboratory equipment, electric light wires, fat catalysts, and agricultural fertilizers. Nickel is known to build up in plants, and when consumed, it is transferred to the human body. Although vital in modest amounts, nickel is harmful to human health in high concentrations. Prostate cancer, laryngeal cancer, nose cancer, and lung cancer are all made more likely by high nickel exposure. The term "heavy metal bioavailability" (nickel) describes how many biological and soil factors affect how well plants can absorb heavy metals. Ni bioavailability in soil is influenced by a number of variables, including soil pH, interactions with other metals, water holding capacity (WHC), cation exchange capacity (CEC), and organic matter (OM). The pH of a solution indicates whether it is acidic or alkaline (degree of acidity). The maximum amount of water that a soil can hold against gravity is known as its water holding capacity. The total amount of cations that can exchange on a negatively charged colloid surface is known as the Cation Exchange Capacity. It serves as a gauge of soil fertility and is related to the availability of nutrients for plants. The amount of CEC is influenced by the material composition, clay mineral type, and soil texture. Total organic carbon refers to the total quantity of carbon bound to organic compounds (TOC). The organic matter in the soil is produced by decomposing plants, animals, microbes, and soil biota. Lower levels of heavy metals are absorbed by plants in heavily polluted soil than in mildly polluted soil. This is due to a larger pH increase brought on by more pollutants and in agreement with a higher KPK, resulting in a stronger soil absorption (Nopjaet al., 2020).

2.5.1 Food chain-based transmission

Plants are a high-risk food source because nickel is highly mobile in them and accumulates readily in their leaves and seeds. As a result, nickel toxicity is correlated with plant uptake, which may have consequences for people and animals further down the food chain (Welch and Cary, 1975).

Plants and nickel: Nickel is a crucial micronutrient for enhanced plant growth, although it is not a necessary element for overall plant growth and development (Brown et al., 1987). Plant absorption is affected by soil pH, organic matter content, and levels of iron-manganese oxide because nickel phytoavailability has been connected to free nickel ion activity in soil solution. According to the Environment Agency (2009), plant species differ in their tolerance and capacity to take up nickel from soils. Plants are more readily able to absorb nickel from anthropogenic sources than nickel from naturally occurring sources. Between 25 and 50 parts per million of nickel are hazardous. While Kabata-Pendias and Pendias (2001) reported phytotoxic concentrations of 40 to 246 ppm DW plant tissue depending on the plant species and cultivars, phytotoxic concentrations of nickel occurred at leaf contents of 10 to 100 ppm depending on the plant species. The most frequently found plants for their high accumulation of nickel and resistance to nickel include cabbage, cauliflower, and turnips as well as Leguminosae like bean and pea. Additionally, Uren (1992) found that nickel poisoning results in chlorosis, decreased root growth, and brown interveinal necrosis in addition to inhibiting plant growth (Kabata- Pendias and Mukherjee, 2007).

Nickel is an essential dietary trace metal, but its functional relevance has not yet been established. This is because numerous animal species are deficient in nickel. Both the function of nickel as a trace element in humans and the absence of nickel have not been established (Scott-Fordsmand, 1997). Human skin's ability to absorb nickel is quite limited, and soil uptake is even more constrained. When radioactive nickel chloride was applied to human breast skin in vitro for 24 hours with and without a spiked reference soil, the mean dermal absorption was 1% with soil and 23% without soil. Contact dermatitis, a highly common and well-known allergic response to nickel in both people and animals, is associated with skin diseases and nickel-induced hypersensitivity (Kabata-Pendias and Mukharjee, 2007).

The primary source of nickel exposure for the general population is food consumption, with secondary sources include air and water inhalation, drinking, oral, and cutaneous routes. Natural levels of nickel in food range from 0.1 to 0.5 ppm, however only a small proportion of foods are contaminated with nickel during production or food processing procedures such leaching from stainless steel, milling flour, or catalytic hydrogenation of fats and oils. Increased consumption of rich food sources of nickel, such as dark chocolate, soy products, dry beans and peas, and oat flour, can increase nickel intake by up to 900 g per person per day or more. According to Cempel and Nikel (2005), nickel's toxic effects come from its capacity to substitute for other metal ions in enzymes, proteins, or bind to cellular compounds. According to Nielsen (1980), nickel interacts with at least 13 essential elements in animals, microorganisms, and plants, including calcium, chromium, cobalt, copper, iodine, iron, magnesium, manganese, molybdenum, phosphorus, potassium, sodium, and zinc. Since nickel carbonyl is linked to systemic effects such moderate nausea, headache, dyspnea, and chest pain; these symptoms may go away or lead to serious pulmonary insufficiency; persistent exposure to nickel oxides and sulphides is therefore linked to an increased risk of lung and nasal malignancies. Nickel-containing mists and dusts can also lead to asthma, pneumoconiosis, and nasal membrane irritation (Kabata-Pendias and Mukharjee, 2007).

2.6 Inorganic and organic amendments

Heavy metal (HM) soil pollution from point sources and diffuse sources is a problem for the environment at large. Mining, inadvertent accumulations, industry atmospheric deposition, energy production, and agricultural chemicals are only a few examples of anthropogenic activities that are significant input channels. This system is regarded as a contaminated environment because soil particles retain a significant amount of contamination. Additionally, there is growing interest in HM pollution because to a constant buildup on top soil and enhanced availability to organisms. As a result, high concentrations of HM and their potential toxicity may affect the ecology of soil, agricultural output, and water quality, raising concerns for both the environment and human health. According to the source, the levels of nickel in serpentine soils exceeded the global standards for human health and the environment.

Heavy metal partitioning in polluted soils is influenced by a number of chemical and biological processes. Metal mobility and bioavailability are decreased by soil sorption, precipitation, and complexation, which all keep HM in place. However, this natural attenuation process can be augmented by on-site chemical technologies, such as organic and inorganic amendments. An economical way to stabilize HM in polluted soil is through chemical immobilization in place. In general, these approaches reduce the availability of HM, increase soil fertility, and promote plant development. While phosphate fertilizers are also effective at immobilizing HM through the creation of stable mineral HM phosphate, organic amendments (compost) contain a high proportion of humified organic matter and may temporarily reduce HM bioavailability in soil. This will allow vegetation to reestablish. As insoluble metal compounds form, they become less mobile through the soil profile and less accessible to the biota pool. Additionally, the simultaneous application of amendments in degraded soil may enhance biological activity and plant growth (Branzini and Zubillaga, 2012).

The physical, chemical, and biological characteristics of soils are all influenced by organic matter, which is a crucial element. It reacts violently with metals and might alter how metals behave in soils. Some of the mechanisms involved in metal retention by organic matter include physical adsorption, electrostatic attraction, hydrogen bonds, and coordination complexation. The solubility and availability of Ni to plants can generally change when organic matter is added to soils. Over time, organic matter in the soil can alter. Numerous processes may have an impact on the retention or adsorption of Ni in soil. Depending on their origin and stability, organic supplements can change the metal solid solution phase partitioning, which in turn changes the metal's bioavailability and environmental fate. Numerous biochemical processes, including hydrolysis, oxidation, and/or depolymerization, cause organic matter to release dissolved organic carbon (DOC). By interacting with the metal ions, this DOC can increase their mobility and bioavailability (Sathya and Mahimairaja, 2015).

2.7 The idea of phytomining

Metals are extracted through a process called phytomining from biomass-rich, hyper-accumulating plant species. Minerals can be removed from the soil safely, cheaply, and easily using the phytomining technique. Numerous plant species behave as hyper-accumulators, holding a lot of minerals in reserve and assisting in their extraction throughout the smelting process. Numerous plant species have the capacity to accumulate large amounts of nickel without suffering any negative effects. One of the plant species that accumulates minerals the most, Alyssum murale favors nickel accumulation. Serpentine soils have a high nickel content in some parts of the world. Serpentine soils have amounts of nickel ranging from 700 to 7000 milligrams per kilogram. According to several studies, the hyperaccumulator plant species Alyssum murale flowers when nickel concentrations are at their highest. More nickel is concentrated in the leaves and flowers than the stem and roots (Kumari et al., 2019).

Due to high capital costs, commercial mining of metals is often conducted from ores that have a high concentration of the desired metals, but these ore deposits are confined to small areas. Sub- or low-grade ore requires a much more room and has much less metal than is required to extract and treat it profitably using conventional techniques. The endemic flora, which contains both constitutive (present in the majority of phenotypic) and adaptive (present only in tolerant phenotypes) mechanisms for metal accumulation and tolerance, is commonly supported by these deposits, which may be found all over the world. The necessity to use such areas to extract marketable metals and generate revenue will increase in the future (Patra and Mohanty, 2013).

Hyperaccumulator plants are used in the process known as phytomining to grow and concentrate a metal. After that, the crop is harvested, and the metal is taken out. Originally, taxa with dry biomass containing >1000 mg/kg (ppm) Ni were considered to be hyperaccumulator plants. This concentration was chosen because it was 100 times higher than the Ni concentration of non-accumulator plants, even when they were growing in ultramafic (also known as "serpentine") soils (Anderson et al., 1999).

Producing a metal harvest from high-biomass plants—which, when burned, provide energy or usable resources—is known as phytomining. A plant species with a high concentration of a desired metal is grown by phytominers, who then harvest the crop and send it to a furnace to burn and extract the bio-ore. As an alternative to destructive mining techniques, phytomining has the potential to change the way metals are extracted from the environment; however, it hasn't yet yielded enough metal to satisfy the world's demand. It is generally agreed that using phytomining is less harmful to the environment than using conventional mining techniques. Phytominers may remove metal pollutants from soil in places with metal-contaminated soil, thereby reviving the soil. However, the land used for agriculture suffers as a result of the extensive plant cultivation. Industrial farming practices deplete the soil, and overgrowing biocrops has the potential to permanently harm an area's ecology.

It is generally agreed that using phytomining is less harmful to the environment than using conventional mining techniques. The health of the soil can be restored by phytominers by collecting metal contaminates from metal-contaminated soil. If production scale is high enough, phytomining could become a less expensive alternative to excavation, although at the moment, large-scale harvesting of plants with metal concentrations is more expensive than getting metals from mines. As metal prices increase and mine yields decrease, this could change in the future. The expenditures of starting a large-scale phytomining farm production would be outweighed by the shortage of metal from mines and the ongoing need for metal by industry. Natural forces decide whether phytomining is successful. Contrary to typical excavation, phytomining depends on factors that affect growth, including weather, altitude, and soil quality. The risks of establishing a long-term phytomining industry in an area rise as a result of changing weather patterns brought on by global climate change. A bad growing season might wipe out an entire crop of metal-producing plants. Much is unknown about the long-term effects of phytomining, as is the case with any new industry. If there was just a little amount of useful farming land available, what other land use would be sacrificed to create room for a mining operation, for instance? The long-term implications of metal-enhanced plants entering the food chain must also be studied by researchers. They must also look for ways to stop metal runoff from the facilities from contaminating the nearby water supply. Much is unknown about the long-term effects of phytomining, as is the case with any new industry. The long-term implications of metal-enhanced plants entering the food chain must also be studied by researchers (Kumari et al., 2019).

3. MATERIAL AND METHODS

3.1. Selection of soils and organic additives for the experiment

3.1.1. Soils

The soils were collected from the surface soil horizon (0-20 cm) from Kazak village, Haskovo region, Bulgaria. The sampling site was located at 41°24'37.5 "N 25°51'58.7 "E (350 m elevation). This region belongs to the Mediterranean climatic influence zone. Winters are relatively mild, and summers are long and hot. The soils are characterised by an acid reaction (pH=6.1) and a low organic matter content (4.58%). In terms of mechanical composition, the soils are characterised as sandy (Table 1). Figure. 1 shows the diffractogram obtained from the X-ray phase analysis. The individual phases are identified based on comparing the obtained intensities with the ICDD-PDF2 powder diffraction standards database. Representatives of several mineral groups are present in the soil samples. Of the primary minerals in the samples, mainly those of the serpentine group (antigorite $(Mg,Fe,Ni)_3Si_2O_5(OH)_4$) are present in an amount of up to 65%, of the talc group (vilemseite $(Ni,Mg)_3Si_4O_{10}(OH)_2$) - 10%, of the amphibole group (ribeykite $Na_2(Fe^{2+}{}_3Fe^{3+}{}_2)Si_8O_{22}(OH)$ - 6%, and quartz (SiO_2)- 11%. The secondary minerals present in the samples can be attributed to the representative of clay minerals - clinochlore ($Mg_5Al(AlSi_3O_{10})(OH)_8$) in an amount up to 10%.

Using the X-ray phase analysis carried out, it was found that the predominant crystalline phase is antigorite and in smaller amounts is present a vilemsite of the talc group. The nickel content of the samples can be explained by the possibility of Ni^{2+} replacing Mg^{2+} in the mineral structure, which is particularly characteristic of those of the serpentine group but is also possible for clay minerals.

Table 1. Main soil characteristics of the tested soil

Index	pH	Organic matter, %	N, %	Mechanical composition, %				
				> 2 mm	2 – 0,02 mm	0,02 – 0,002 mm	<0,002 mm	<0,02 mm
Kazak	6.1	4,58	0.12	17,3	62,6	8,6	11,5	20,1

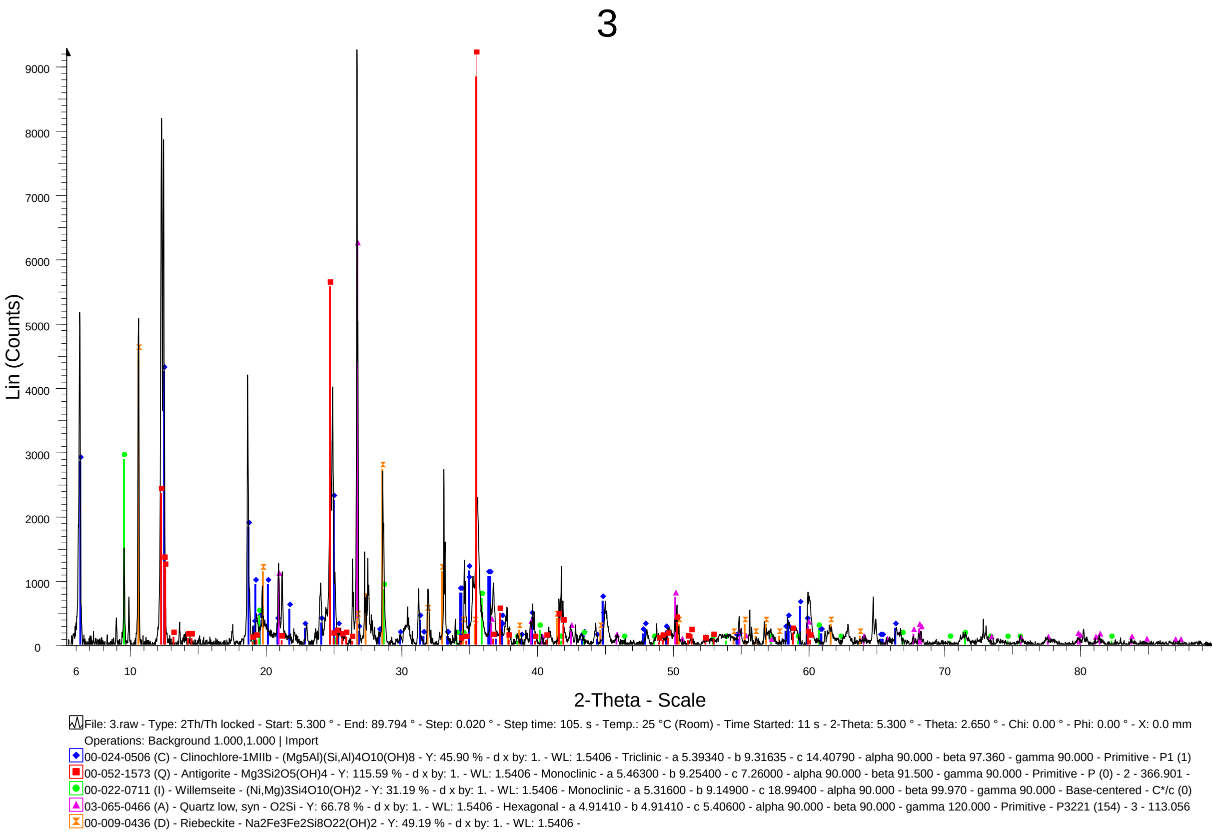


Figure. 1. Diffractogram of a soil sample from the area of the village of Kazak

3.2. Organic additives

Organic soil additives were tested within the framework of the study. From the organic amendments, compost and vermicompost obtained from the processing of natural manure and other organic wastes from California worms were selected. Biochar and activated carbon were also tested.

Biochar is a carbon-rich solid material produced by pyrolysis of biomass (manure, sewage sludge, woody biomass, and crop residues) in an oxygen-limited environment. Biochar is added to soil to improve its physical properties, such as water and nutrient retention, and enhance plant growth. The effects of their addition to soils depend on many factors, including the properties of the raw material and processing conditions, soil type, environmental conditions and crop type. Research on the effects of biochar on plant yield and quality is often controversial. Positive, negative, and sometimes neutral effects on crop yield and quality are reported.

Activated carbon is a carbon-rich solid that is obtained from biomass or other carbonaceous substances, such as coal or tar, by pyrolysis. In this process, the carbonaceous material is also 'activated' by strategies that significantly increase the surface area of the material, allowing it to capture (or 'adsorb') a more significant amount of molecules. This high adsorption capacity allows activated carbon to be effective in removing pollutants from water and air, which is why activated carbon is commonly used for remediation or purification and enhances plant growth.

Biochar and activated carbon have similar chemical compositions. They all contain many 'aromatic hydrocarbons' which are strongly bound together, making them stable and suitable for application to the soil to improve plant growth.

Table 2 presents the chemical properties of some of the organic additives used. Biochar is characterised by a very high pH (9.6), the typical value for most biochar, and high EC values (3.6). The total carbon content (67.7%) and the organic carbon content (66.7%) are within the thresholds of the European organic coal certification and according to IBI-Standard Class 1. The molar ratio H/organic (2.36/66.7) meets the requirements of EBC and IBI-Standard (H/Corg ≤ 0.7) (data not shown in table). Lower H/Corg ratios indicate an indication of long-term stability and persistence of biochar in the soil contributing to carbon sequestration. In contrast, H/Corg values above 0.7 are an indication of the shortcomings (improper conduct) of pyrolysis. Vermicompost and composts are mature and stable in chemical characteristics (Table 2). In contrast to biochar, they have a neutral or slightly alkaline reaction and are higher in N.

Table 2. Agrochemical characterization of organic additives used

Organic additives	pH (H_2O)	Org.Matter, %	Total N, %	P, mg/kg	K, mg/kg	Ca, mg/kg	Mg, mg/kg	Zn, mg/kg
Compost	7.5	53.80	1.81	1005	2452	420	168	93.2
Vermi-compost	7.8	34.94	1.96	1009	5684	893	410	191.8
Biochar	9.6	66.74	1.48	8000	16000	12000	600	150

3.3. Experiment

Vegetation pot trials were conducted on serpentine soils from the area of the Kazak village. According to the following scheme, the experiments were set in 0.25 l containers with dimensions 7x7x8 cm, in 9 treatments with three repetitions. 2. Soil + compost (2.5%). 3. Soil + compost (5%). 4. Soil + vermicompost (2.5%). 5. Soil +

vermicompost (5.0%). 6. Soil + biochar (2.5%). 7. Soil + Biochar (5.0%). 8. Soil + activated carbon (2.5%) and 9. Soil + activated carbon (5.0%).

The soils were sieved through a 2 cm^2 mesh sieve. The tested organic soil amendments (compost, vermicompost, biochar and activated carbon) were added at 2.5%, and 5.0% (recalculated based on soil dry weight) and carefully mixed with the soil by hand. The total amount of soil used in all treatments was 150 g. In the treatments of the control experiment, the soil was placed directly into the containers, while in the treatments with the organic amendments, the tested amendments were added to the soil in an amount of 2.5%, and 5.0% (recalculated based on soil dry weight) and carefully mixed with the soil by hand. The soil moisture content was maintained at 75% of the permanent field humidity. After a 4-week incubation period, five seeds of the test plant Odontarrhena chalcidica were sown in each pot. The containers with the plants were placed in a growth chamber called Growarm 100 propagators, which provide a suitable constant temperature and humidity for seed germination and plant growth. The propagator has an 8W heated base which slightly heats the seed containers, accelerating germination. The base is fitted with a capillary irrigation pad that retains water and ensures it is evenly distributed, preventing the seed dishes from drying out or over-irrigating. The transparent lid is designed to handle any condensation and features two sliding vents to help control humidity and temperature. Maxibright LightWave T5 luminescent lamps were installed above the propagators to provide the necessary lighting. The blue-white colour spectrum of the lights is suitable for seedling development and the early growing season. The T5 tubes are mounted in a sturdy metal box with a reflector that ensures the light is directed from each tube down to the plants where it is needed. Due to the reduced heat radiation, they were placed closer to the plants. After six days, seed germination was recorded from the supplemented treatments, and on day 10, seed germination was recorded from the control. Plants were watered every other day, and weeds from the supplemented treatments were removed by hand. In some of the pots, the number of germinated seeds was more than one, so thinning was done, and one plant per pot was left.

Harvesting took place 90 days after sowing. After being photographed, the plants were cut one inch above the soil, washed with distilled water and placed in labelled paper bags. Fallen leaves on the soil were also collected and added. Fresh and dry biomass yield was recorded. The plants were dried in a dryer at 80°C for 24 hours and the biomass was weighed. After removing plant roots, soils were air-dried and sieved through a sieve (< 2 mm) and stored in paper bags for subsequent analyses.

3.4. Methods used in sample preparation for analysis

3.4.1. Soil samples

Determination of basic soil characteristics

To determine the influence of organic amendments, soil samples were taken for analysis one month after application to the soil. Pre-dried soils sieved through a 2 mm sieve were analysed for soil pH (ISO 10390), total nitrogen by the Kjeldahl method (N Kjeldahl), and total organic matter (BDS EN 13039). The content of total (extracted with aqua water, according to ISO 11466) and DTPA-extracted mobile forms of heavy metals, micro and macro elements in the soils was determined before the additives' application and four weeks after the application of the soil organic amendments.

The same procedures were applied to the organic amendments.

1. Determination of the total element content of the soils

In preparing the samples for analysis for trace elements and toxic metal content, the procedure described in the well-established international standard ISO 11466 was used: 3 g of air-dry soil was weighed to the nearest 0.001 g and moistened with 1 cm_3 H_2O. Add 28 cm^3 of HCI: HNO_3 mixture (3:1). The sample is left for 16 hours at room temperature. Place on a sand bath and heat for 2 hours at 180 to 200^o C. Quantitatively transfer the residue to a volumetric flask by filtering through a blue band filter and bringing the volume to 50 cm3. In addition to good reproducibility of the results, this method allows correct comparison of the results obtained with other authors' results.

2. Determination of available (mobile) forms of heavy metals, micro and macro elements in soils

The content of nutrients and mobile forms of heavy metals was determined by extraction with DTPA (ISO) solution. This method was used to determine the mobile forms of K, Ca, Mg, Zn, Fe, Mn, Cu, and toxic metals (As, Se, B, Cd, Ni, Pb, Mo). The method's main advantage is the simultaneous determination of heavy metals and macro and trace elements in the extract by ICP-AES.

Weigh 10 g of soil sieved through a 2 mm mesh sieve into a 125 cm^3 Erlenmeyer flask and add 20 cm^3 of DTPA solution. Place the flask on a shaker and shake for 120 minutes, then filter. In the filtrate, the content of mobile forms of heavy metals, macro and trace elements is determined.

3. Determination of the effective cation exchange capacity (CEC)

The CEC was measured according to a modified version of ÖNORM L 1086-89 (Austrian Standards, 2014). 100 ml of a 0.1 M $BaCl_2$ solution (unbuffered) was added to 5 g of air-dried soil (< 2 mm) and stirred to ensure that all soil was in contact with the solution. Samples are left overnight and placed on a shutela for 2 hours at 20 rpm at room temperature. Allow the soil particles to settle for 20 minutes before filtering. The filtrates were acidified (2 % HNO_3), and the contents of Al, Ca, Fe, K, Mg and Na were measured using ICP-OES (inductively coupled plasma - optical emission). The rest was stored in the freezer at - 20°C. CEC was calculated by summing the soil's Ca, , and Mg contents in mmolc/kg.

4. Determination of the mineral composition of soils

X-ray phase analysis was used to determine the mineral composition of the soils. Powder X-ray diffraction patterns were recorded in the angular range of 5.3 to 80o2θ with a constant step of 0.02 o2θ on a Bruker D8 Advance diffractometer with Cu Kα radiation and a LynxEye detector. Phase identification was performed with the Diffracplus EVA program using the ICDD-PDF2 database. Phase quantification by the Rietveld method was performed with the software package Topas-4.2. The accuracy of the quantifications was 3-5%.

5. Determination of total nitrogen content in the soil

In the determination of total nitrogen content in the soil, sample preparation for analysis was carried out according to the classical Kjeldahl method, which has undergone various changes in temperature and decomposition time, acid concentration and oxidising catalysts. The principle of the method is based on the combustion of organic matter in the soil and the reduction of nitrate by phenol or salicylic acid in the presence of catalysts, whereby the nitrogen contained in the soil is converted to ammonium sulphate. The resulting ammonium sulphate is decomposed by alkalinising the solution with concentrated NaOH. The free ammonia separated is distilled off and combined with boric acid of known concentration, the excess of which is titrated with 0.2 N HCl. The method of operation was modified and proposed for use by the manufacturer, VELP Scientifics. Depending on the nitrogen content, the samples to be analysed are in quantities of 0.5 g to 2 g for soil samples and 0.25 g to 1 g for plant samples.

Procedure: weigh the appropriate sample, add 7 g K_2SO4, 5 mg Se, 7 cm^3 k.H_2SO_4 and 5 cm^3 H_2O_2 (hydrogen peroxide). The samples thus prepared are placed on the digestion apparatus, which is pre-programmed for digestion at a temperature of 420^0C for 20 minutes. After decomposition, the sample is distilled by pre-alkalising with the addition of 35% NaOH. The resulting distillate was collected in a flask with 4% boric acid. The excess was titrated with 2N HCl in the presence of Toshiro's indicator reagent. The resulting amounts of hydrochloric acid consumed are used to calculate the total nitrogen.

6. Determination of soil organic matter

Organic matter influences many of the physical, chemical and biological properties of soils. Some of the properties influenced by organic matter include soil structure, soil compressibility and shear strength. In addition, it also affects the water holding capacity, nutrient contributions, biological activity, and water and air infiltration rates (Zebarth et al., 2015). The mass of an empty, clean, and dry Beaker (M_B) was determined and recorded. A part of or the entire oven-dried test specimen from the moisture content experiment was placed in the beaker and determine and record the mass of the beaker and soil specimen (M_{B+S}). The beaker was placed in a muffle furnace. Gradually the temperature was increased in the furnace to 440^0C. The specimen was left in the furnace overnight. The beaker was removed carefully using the tongs (the beaker was very hot), and allowed it to cool to room temperature. The mass of the beaker containing the Burned/Ash soil (M_{AS}) was determined and recorded. The beaker was emptied and cleaned.

(1) To determine the mass of the dry soil.

$M_D=M_{B+S}-M_B$

(2) To determine the mass of the ashed (burned) soil.

$M_A=M_{AS}-M_B$

(3) Determine the mass of organic matter

$M_O = M_D – M_A$

(4) Determine the organic matter (content).

$OM = (M_O/M_D)*100$

3.4.2. Plant samples

Mineralisation and analysis of plant samples for macro-and trace element content. The heavy metal content of aboveground mass of plant sample was determined by dry washing and microwave mineralisation methods. EPA Method 3052: Suitable for sample preparation for atomic absorption determination of heavy metals. A Milestone 1200 MEGA microwave system with a 10 MRD 300 rotor (10 positions) was used. 0.5 g of the dried and well homogenised sample was weighed to the fourth digit and transferred to Teflon containers, after which 8 cm^3 of hydrochloric and nitric acids were added to each in a 3:1 ratio. The mineralisation program includes three stages: (i) 5 minutes of 250 W non-pulsating irradiation; (ii) 5 minutes of 400 W pulsing irradiation; and (iii) 5 minutes of 600 W pulsing irradiation. Cool the sample after ventilation for 1 minute, and transfer quantitatively to a 25 cm3 volumetric flask. A blank was also included in each series of mineralised samples by the methods described to assess the purity of the reagents and glassware used.

3.5 Apparatus used

- Mineralisation of the samples

A Milestone 1200 MEGA microwave system with a 10 MRD 300 rotor (10 positions) was used in the mineralisation of some of the soil and plant material samples. The maximum power is 1000 W and the maximum pressure in the Teflon vessels is 30 bar.

- Quantifications

An atomic absorption spectrometer "Analyst 200" by Perkin Elmer and an atomic emission spectrometer ICP-AES by Jobin Yvon Emission - JY 38 S, France were used to determine the macro-and trace element contents of soil and plant samples. The accuracy of the measurements was assessed by comparing the results obtained with the consensus values in the certificates of the reference materials used.

3.6. Statistical processing of the results

SPSS for Windows was used in the statistical processing of the data. Correlation-regression analysis was performed between pH, organic matter content, and mobile forms of nickel in soil and the concentration of the elements in the tested plant.

4. RESULTS AND DISCUSSION

4.1 Soil characteristics

The studied soils are located in the eastern part of the Rhodope Mountains. The soils of this study show characteristics typical of serpentine soils. They contained high concentrations of total Ni, a high Mg to Ca ratio, and low contents of the macronutrients nitrogen, phosphorus, and potassium (Cerdeira-Pérez et al. 2018). The main minerals in Cossack soils are antigorite of the serpentine group, typical of soils that develop on ultramafic rocks, and quartz. Talc is a typical weathering product and occurs in soils as a willemsite. Serpentine is the most common Ni-bearing phase in Cossack soils and talc. Ni bioavailability is high in soils with amphibole, talc, chlorite, serpentine, pyroxenes, goethite and other Ni-bearing phases (Echevarria et al. 2005). Because Ni availability depends on components such as organic matter and clay minerals that serve as exchangers for Ni, young soils with high soluble Ni content may have poor bioavailability due to the lack of these components (Echevarria et al. 2005). Microbial activity and soil pH conditions influence Ni availability by altering the stabilisation of Ni-containing mineral phases of the metal.

Serpentine soils from Kazak village are characterised by elevated levels of the metals Ni, Cr, Co and Mn, which is typical for such soils (Table 2). The total Ni content in the studied soil reached 1597.3 mg/kg, while DTPA extracted Ni reached up to 161.9 mg/kg. The Ni concentration was much higher than that considered toxic to normal plants by Allen et al. (1974) and Kabata-Pendias (2001).

The Cr content in soils reaches 2060.6 mg/kg and exceeds the upper limit given for normal soils by Allen et al. (1974) and Brooks (1987) (500 mg/kg). The values we obtained are similar to those found by Karataglis et al. (1982) for northern Greece and Bani et al. (2007) for Albania.

Cobalt in soils ranges up to 413 mg/kg and is within the normal range for serpentines. The relatively easy interaction of Co with all metals that are geochemically or biochemically associated with Fe has a significant influence on its behaviour in soils and on its phytoavailability.

The Fe content in serpentine soils is high and reaches 93194.5 mg/kg, which is typical for serpentine soils of the Balkan Peninsula (Pavlova, 2001). Brooks and Yang (1984) found that the concentration of Mg in plant tissues is inversely proportional to the concentration of other nutrients such as Fe, Co, and Mn. These data suggest that

Mg uptake leads to less uptake of other nutrient elements. Brooks and Young (1984) proposed that elevated Mg levels in serpentine soils and antagonism with other elements may be the most important factor in serpentine syndrome.

The Mn content reached up to 2597.3 mg/kg. Iron content in the studied soils shows typical values for serpentine soils reaching up to 93194.5 mg/kg (based on literature data from Bani et al., 2007 - 2.43-6.28%)

The Cu and Zn contents reach 6.9 and 81.7 mg/kg, respectively. These values fall within limits for normal soils (Allen et al., 1974; Kabata-Pendias, 2001). Serpentine soils are rich in Mg but low in Ca. Mg contents reach up to 60575.5 mg/kg and calcium up to 6592.7 mg/kg. A low characterises the soil Ca/Mg ratio (0.11), leading to Ca deficiency stress for plants.

4.2. Influence of organic amendments on basic agrochemical properties of soil

The effect of the applied organic amendments on the main soil parameters is shown in Figure 2. The results we obtained show that soil's chemical and physical properties are affected by the type and amount of soil amendments.

- **pH**

Soil pH is an important soil characteristic that gives a general idea of the plant growth and development environment and the behaviour of macro-and trace elements. Soil pH is the main factor influencing the availability of metals in the soil and thus the uptake of metals by plants. It is known that pH values in serpentine soils vary widely, mainly ranging from slightly acidic (6.1) to alkaline values (8.8) (Brooks 1987; Echevarria 2018). In this study, soils from Cossack had a pH of 6.1, i.e. slightly acidic, which may account for the higher values of plant available trace elements such as Fe and Mn (Babalonas et al. 1984). After introducing organic additives in the cultivation of Odontarrhena muralis, the pH values increased. From the results presented in Figure. 2, it can be seen that the pH values varied widely depending on the type of the amount of meliorant applied. The application of organic additives increases soil pH (Figure. 2), depending on the amount of additive used. In general, the application of additives results in a change in soil pH to slightly alkaline values. In serpentine soils, an increase in pH can decrease the Ni content of the aerial parts of hyperaccumulator plants. Kukier et al (2004) found the maximum uptake of Ni by the hyperaccumulator Alyssum at pH 6.4, with significantly less Ni uptake with increasing pH.

Biochar has specific properties that correct soil acidity due to its alkaline nature and high buffering capacity. Our results confirm the findings of Jien and Wang (2013) and Yang et al. (2016) that the addition of biochar to soils leads to an increase in soil pH, with biochar dosage being influential. E.g., it was found that soil pH increased with an increasing amount of added rice straw biochar, with fine rice straw biochar being more effective in increasing soil pH. However, the addition of bamboo biochar did not result in a significant difference in pH values.

Very often, however, results in the literature are contradictory, as higher availability of Ni in soil at a lower pH value does not always result in higher uptake by plants. According to Kaupenjohann and Wilcke (1995), in serpentine soils with low organic matter content Ni is strongly bound to iron oxides, which are chemically very inactive. The pH value leads to a change in charge of iron oxides and the sorp tion properties of clays. At low pH values, the sorption of metal cations onto iron oxides is very low and increases rapidly at a pH value of 6.5. In soils with a very high organic matter content, the opposite effect is observed, since at higher pH metal-humic complexes dissolve and Ni becomes more soluble (Herms and Brümmer 1983). According to Nkrumah et al (2016), the optimum range determined for phytoextraction of Ni for hyperaccumulator species is pH 5 - 6.5.

The significant increase in pH was also observed in the study of Cerdeira-Pérez et al (2019) when growing Odontarrhena muralis and Odontarrhena serpyllifolia on serpentine soils. According to Mitton et al. (2016), the increase in pH can be explained by the release of rhizodeposits from the decarboxylation of organic anions during plant growth. According to Zhang et al. (2018), the reason for this is that plant roots contain 30 - 60% of net photosynthetic carbon. In general, rhizosphere processes are thought to be responsible for the increase in pH, but so far there is no definitive answer to the question of how these processes occur in hyperaccumulator plants.

The results of this study indicate that pH affects the mobile forms of metals. Significant negative correlations were found between pH and DTPA- available forms of Ni (r = 0.893).

- Exchangeable cations and cation exchange capacity (CEC)

The amount of exchangeable ions was determined by extraction with 0.1 M BaCl2 solution. Introduction of organic additives resulted in an increase in the amount of exchangeable cations Ca-$BaCl_2$, Mg-$BaCl_2$ and K- $BaCl_2$.

Ca (Ca-$BaCl_2$)

Ca amounts varied considerably between treatments, influenced by the type of additive and dose. The highest Ca concentrations were recorded when 5% vermicompost was applied. The lowest values were recorded in the control, followed by the activated carbon, biochar and compost application treatments. The amount of exchangeable ions increased in direct proportion with increasing doses of applied compost and vermicompost. However, for activated carbon and biochar the opposite trend is observed.

Mg (Mg-$BaCl_2$)

The amounts of Mg varied considerably between treatments, influenced by the type of additive and dose. The highest concentrations of Mg were recorded for the 5% compost application. The lowest values were recorded in the control, followed by the vermicompost, activated carbon and biochar application treatments. The amount of exchangeable ions increased in direct proportion with increasing dose of compost applied. A similar but less pronounced relationship was observed for the vermicompost variants. However, the opposite trend is observed for activated carbon and biochar.

Ca:Mg ratio

In serpentine soils the Ca:Mg ratio is usually low and less than 1 (Ca:Mg < 1). A low Ca:Mg ratio is often associated as one of the limiting growth factors of serpentine soils (Brooks, 1987), but hyperaccumulators are naturally adapted to low Ca:Mg ratios. Typically, higher Ca:Mg ratios are associated with better soil texture, soil slope, and water infiltration rates, while Ca:Mg ratios below < 2 may indicate a need for Ca fertilisation (e.g., lime, gypsum) (Schulte and Kelling, 1985).

The introduction of organic additives influences the Ca:Mg ratio. The Ca:Mg ratio was less than 1 in control and the variants with compost, activated carbon and biochar application, except for vermicompost application (Ca:Mg ratio > 1). The Ca:Mg ratio remained almost unchanged with compost and activated carbon application, decreased with biochar application and increased to 1.22 -1.34 with vermicompost application. The Ca:Mg ratio values depend on how much the Mg content increases relative to Ca. Mg increases more than Ca in the biochar variant, whereas the opposite trend is observed with vermicompost. However, according to some authors (Schulte and Kelling, 1985), the Ca: Mg ratio can be a misleading indicator if the amount of Ca and Mg in the soil is not also taken into account. For example, a low Ca: Mg ratio may be due to low Ca levels, but normal Mg levels or sufficient Ca concentration but elevated Mg content. In addition, the amount of Ca and Mg in the soil may be insufficient but still, have a favourable ratio.

K (K-$BaCl_2$)

The amounts of K varied considerably between treatments, influenced by the type of additive and dose. The highest K concentrations were recorded in the biochar and 5% compost treatments, and the lowest in the activated carbon treatments. Lower values compared to the control were also found with the application of vermicompost. The amount of exchangeable K ions increased in direct proportion with increasing dose of compost applied. In the other treatments, the amount of additive did not significantly affect the exchangeable K ion content.

- Cation Exchange Capacity (CEC)

The soil's cation exchange capacity indicates the amount of exchangeable cations in relation to a given mass of soil (kg soil). The effective cation exchange capacity (CEC) is determined by the sum of the charge of the major exchangeable cations (Ca^{2+}, Mg^{2+}, K^{+}) at the current soil pH value. Organic additives influence the soil CEC. The application of organic additives leads to an increase in the soil CEC, the dose being influential. As the amount of compost and vermicompost applied increased to 5%, the CEC increased to 21.99 mmolc/kg and 18.98 mmolc/kg, respectively, compared to the control (13.99 mmolc/kg). The application of activated carbon and biochar also increased soil CEC, but this increase was more pronounced at the lower rate (2.5%).

According to some authors, biochar application leads to an increase in soil CEC (Van Zwieten et al., 2010; Agegnehu et al., 2016), while according to others, the addition of biochar does not affect soil CEC (Méndez et al., 2012; Xu et al., 2014). E.g. Jiang et al. (2012), soil CEC increased from 3.55 in control to 4.90 and 6.12 cmol/kg when 3% and 5% biochar was applied. Similar results were also found by Ghorbani et al. (2019), according to which CEC of loamy sandy soil increased significantly by 20% and 30%. As a result of the addition of 1% and 3% biochar, this increase was significantly lower (9% and 19%) in clay loam soil. According to Baumgartner et al. (2017), soils with a pH between 6.0 and 6.5 and an average bulk density should show an effective CEC between 170 and 180 mmolc/kg. Application of limiting additives leads to an increase in CEC, and values in this range have been found for compost, biochar and vermicompost applications. Similar results were obtained from the application of organic amendments (Thuringer, 2020), while inorganic fertilisation (NPK) and control had CEC below this threshold. Our results are consistent with Ghasemi et al. (2018), who reported lower CEC after NPK application and significantly higher CEC in plots treated with manure. Álvarez-López et al. (2016) reported 66 mmolc/kg CEC in untreated soils, 81 mmolc/kg when applied with NPK, while CEC increased to 238 mmolc/kg in soils to which compost was added.

- Soil organic matter (SOM)

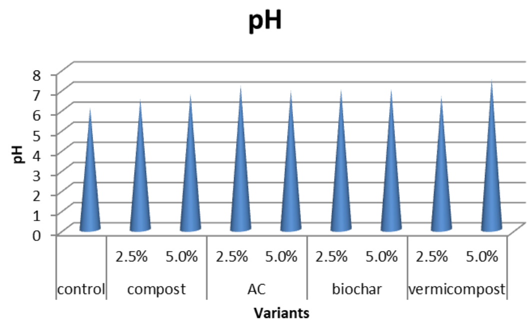
Soil organic matter plays an important role in soil due to its high cation exchange capacity as well as the ability to form complex compounds. Soil organic matter is considered as an additional source of nutrients (Sparks, 1995). Many of the biological and physicochemical properties of soil are controlled by soil organic matter (Doran and Perkin, 1994) as it improves several soil parameters such as structure, biological activity, water holding capacity, nutrient retention, and filtering and buffering functions (Baumgartner et al., 2017). Thus, soil organic carbon/organic matter is essential for maintaining soil productivity and fertility (Baumgartner et al., 2017). The amount of carbon/organic matter is often closely related to soil texture, as soils dominated by smaller pore sizes (e.g. clay, silt) often exhibit higher humus content (Shukla et al., 2006).

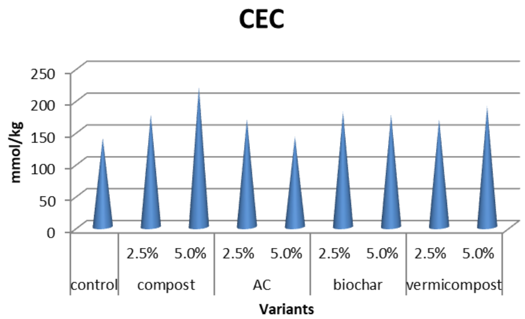
The total organic matter content of the control soil reached 4.58%. Total nitrogen content was low and reached up to 2.8 g/kg. The C: N ratio was high, confirming the infertility of these soils.

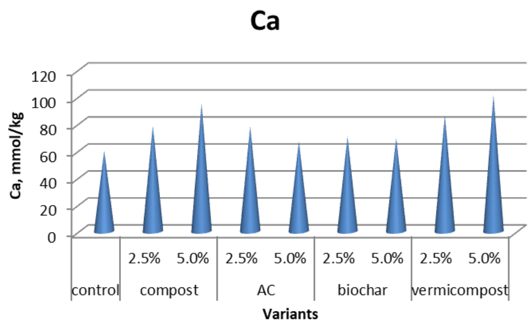
The increase in soil organic matter with organic amendments is due to the high organic matter content of compost and vermicompost, activated carbon and biochar (Table 2). The application of organic amendments resulted in a significant increase in soil organic matter content compared to the initial level (Figure. 2). Soils treated with compost, vermicompost, activated carbon, and biochar had significantly higher organic matter content than the control. Our results show that organic matter increased proportion to the amount of activated carbon and vermicompost applied. No such trend was observed for compost and biochar.

The organic matter content reached 35.97 and 7.43% when 2.5% and 5% vermicompost were applied. The incorporation of activated carbon increased the organic matter content to 8.49 and 10.78 (Figure. 2).

The application of organic amendments increased the organic matter content of the soil and improved its structure. According to Fortuna et al. (2003), the addition of vermicompost can increase the carbon content by up to 45%, compared to the initial levels, thus leading to an increase in the stability of soil structures, particularly of macroaggregates. Biochar is a C-rich solid material obtained by pyrolysis of biomass. As an organic carbon material, the addition of biochar to soils increases the organic matter content. Therefore, adding biochar as an amendment to soils with low fertility can be particularly effective in increasing the CEC content and improving soil quality (Luo et al., 2017). Increases in soil organic carbon content resulting from biochar addition have been reported by several authors (Xu et al., 2014). Ajayi and Horn (2016) found that biochar addition improved total carbon content. The authors suggested that this may be due to the added biochar's sorption of organic matter and nutrients. Ippolito et al. (2016) found that biochar application in vascular trials increased soil organic carbon content up to six times compared to the control, with this increase being directly proportional to the applied rate (0, 1, 2 and 10%).







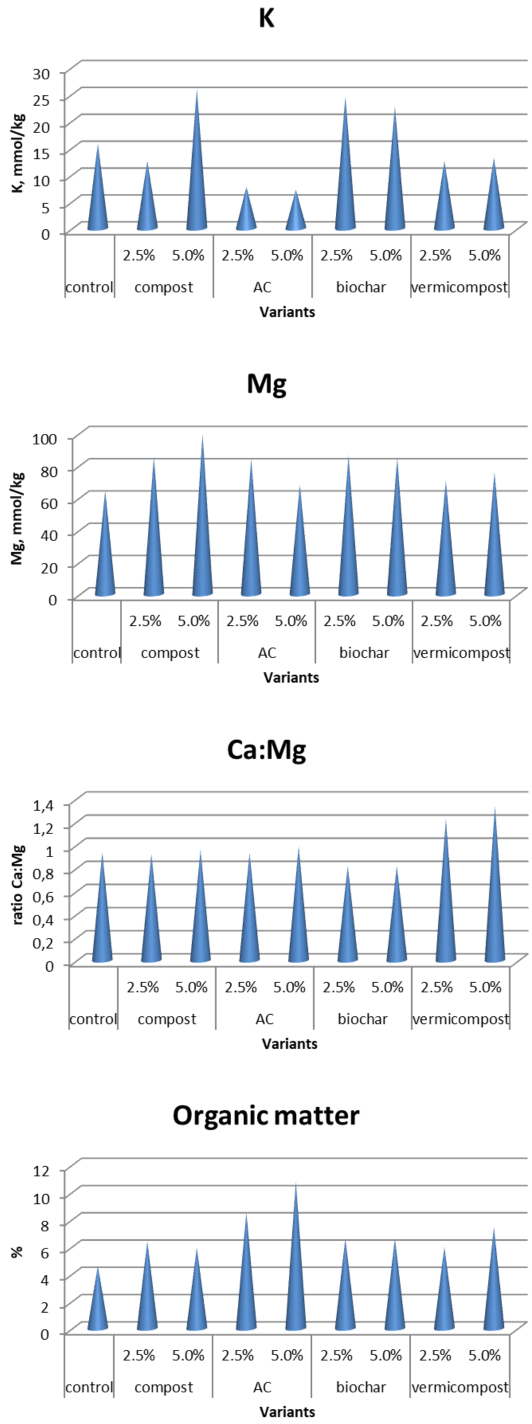


Figure.2. Effect of organic additives on pH, CEC, organic matter and concentrations of Ca, Mg and K and Ca:Mg ratio

4.3 Effect of organic amendments on mobile forms of Ni, macroelements (P, K, Ca and Mg) and trace elements (Cu, Fe and Mn) in soil

The deficiency of essential nutrients (such as N, P and K) characteristic of serpentine soils creates unfavourable conditions for plant growth. For plant development, it is necessary to improve the soil's nutrient supply to maximise the efficiency of metal uptake by the hyperaccumulators. In our experiment, organic matter was added to improve soil characteristics.

- Nickel (Ni)

The mobile forms of the metals were measured using DTPA extraction (diethylene triamine pentaacetic acid). The amount of Ni extracted from the DTPA solution represents Ni that is exchangeable and complexed to the surfaces of organic matter and clay minerals and is therefore available for plant uptake (Echevarria, 2018).

The application of organic additives leads to a decrease in the soil's bioavailable Ni (DTPA) content, and this decrease is more pronounced at 5% additive application in all treatments. The most significant decrease was observed with the application of 5% vermicompost (77.4 mg/kg) (Figure.3).

A significant negative correlation was found between organic matter content and DTPA-Ni (0.71 mg/kg). Soils with higher organic matter content had lower DTPA-Ni values. The lower DTPA-Ni content may be explained by the higher metal holding capacity of soils as a result of increasing organic matter content, where more significant binding of cations to negatively charged surface or organic ligands was observed. Our results are consistent with Álvarez-López (2016), who reported a decrease in extractable Ni after compost addition.

The mobile forms of Ni are influenced by soil texture, pH, electrical conductivity (EC), and organic matter (Massoura et al., 2006; Chardot et al., 2007; Kabata Pendias, 2010). Massoura et al. (2006) and Chardot et al. (2007) reported a significant positive correlation of Ni-DTPA with exchangeable Ca and CEC and pH, clay content, total Mg and Fe, exchangeable Mg and free Fe oxides. However, Chardot et al. (2007) and Alves et al. (2011) found no significant correlation between DTPA-extractable Ni and soil carbon content.

Our results show that DTPA-Ni decreases with higher CEC and Ca values, The addition of compost leads to a reduction of available Ni in the soil, which is consistent with the results of Thuringer (2020). This can be partly, but not entirely, explained by soil dilution and the formation of organometallic insoluble complexes that reduce the bioavailability of metals in soil (Mench et al., 2000; Vangronsveld et al., 2009). The effect of compost addition on the available forms of Ni in soil reinforces the need to optimise the amount of addition to maximise biomass productivity, minimise the reduction of plant-available forms of the metal, and prevent reduced uptake and accumulation of the metal in plants.

- Phosphorus (P)

Soils treated with compost and vermicompost contained significantly more P than control soils (Figure 3). The amount of applied ameliorants also has an effect. The application of compost and vermicompost resulted in an increase in soil P content, which is more pronounced when 5% compost (4.25 mg/kg) and 5% vermicompost (4.85 mg/kg) was applied. The application of activated carbon at both rates resulted in a decrease in P content. In contrast, the application of 2.5% biochar resulted in a slight increase to 3.0 mg/kg, and a decrease was observed with the application of the 5% amendment.

The increase in DTPA-extracted P after compost and vermicompost application may be due to the release of humic acids during the decomposition of organic matter, resulting in the conversion of non-digestible forms of phosphorus to digestible forms.

- Potassium (K)

DTPA-K increased significantly with the application of compost and biochar, and this increase was more pronounced with the application of the 2.5% amendment (Figure. 3). The application of activated carbon resulted in a significant decrease in K to 13.7 mg/kg, while the application of vermicompost resulted in a slight decrease in K to 99.4 mg/kg and 121.6 mg/kg.

- Calcium and magnesium (Ca and Mg)

The application of 2.5% compost, 2.5% biochar and 2.5% vermicompost did not affect the amount of Ca in the soil, while the application of 5% additive resulted in a decrease in its content. The application of activated carbon resulted in a decrease in Ca content to 479.8 mg/kg and 522.6 mg/kg, respectively.

The application of vermicompost resulted in a slight decrease in soil Mg content, with no effect on the amount of additive applied. Biochar increased Mg content, with this increase being more pronounced when a 2.5% additive was used. The application of 2.5% compost and activated charcoal did not affect the amount of Mg in the soil, while a decrease was observed with 5%.

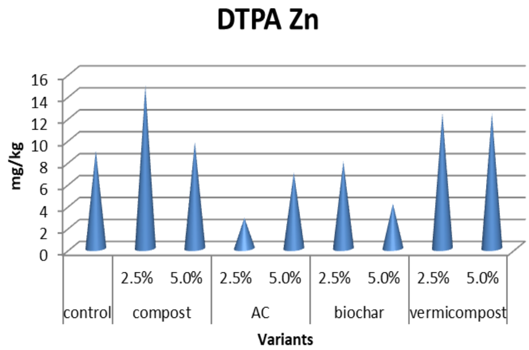
- Copper, zinc, iron and manganese (Cu, Zn, Fe and Mn)

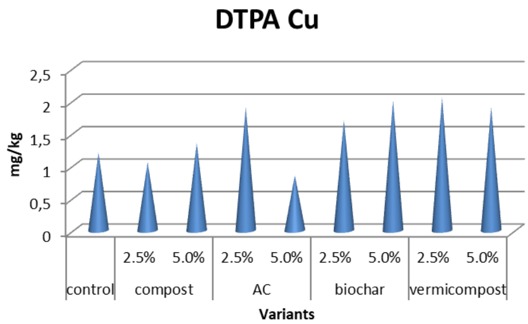
The application of biochar resulted in an increase in the Mn content of the soil compared to the control, this increase being more pronounced at 5% application (489 mg/kg). The application of biochar at both doses resulted in a decrease in Mn content, with no effect on the amount of additive applied. The application of 2.5% compost and vermicompost increased Mn content, while a decrease was observed with a 5% supplement.

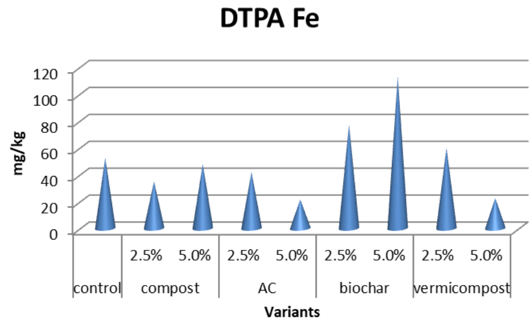
The imported amendments significantly affected the Fe mobility (Figure 3). The application of biochar increased soil Fe content, which is more pronounced at 5% amendment (113.1 mg/kg). Application of 5% activated carbon, and 5% vermicompost resulted in a decrease in Fe content to 21.5 mg/kg and 22.55 mg/kg, respectively.

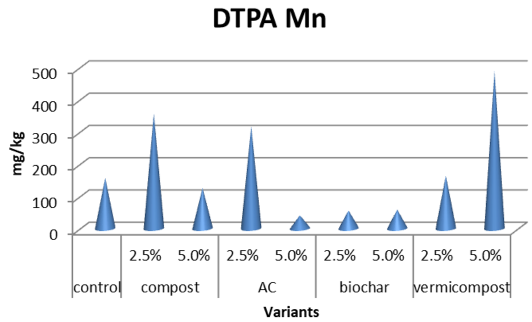
The application of compost, biochar and vermicompost increased the Cu content of the soil, while the opposite trend was observed for activated carbon.

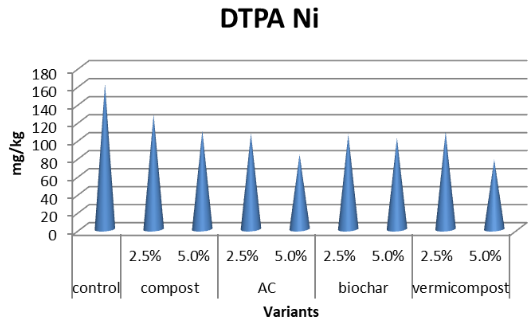
The application of vermicompost resulted in an increase in the content of mobile forms of zinc up to 12.35 mg/kg. In contrast, the application of activated carbon and biochar resulted in a decrease. The application of compost leads to an increase in the zinc content of the soil, with a more significant increase observed in the variant with the application of 2.5% compost.

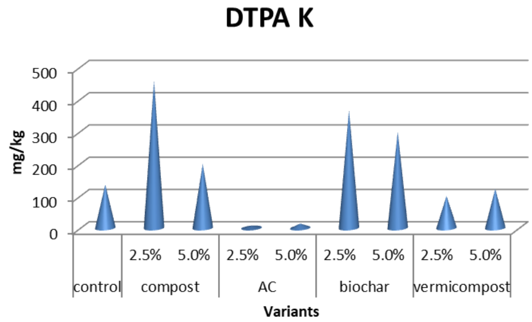


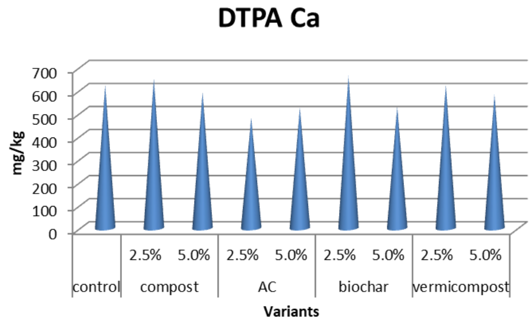



DTPA Fe
mg/kg
120
100
80
60
40
20
0
2.5% 5.0% 2.5% 5.0% 2.5% 5.0% 2.5% 5.0%
control compost AC biochar vermicompost
Variants


DTPA Mn
mg/kg
500
400
300
200
100
0
2.5% 5.0% 2.5% 5.0% 2.5% 5.0% 2.5% 5.0%
control compost AC biochar vermicompost
Variants


DTPA Ni
mg/kg
180
160
140
120
100
80
60
40
20
0
2.5% 5.0% 2.5% 5.0% 2.5% 5.0% 2.5% 5.0%
control compost AC biochar vermicompost
Variants


DTPA K
mg/kg
500
400
300
200
100
0
2.5% 5.0% 2.5% 5.0% 2.5% 5.0% 2.5% 5.0%
control compost AC biochar vermicompost
Variants


DTPA Ca
mg/kg
700
600
500
400
300
200
100
0
2.5% 5.0% 2.5% 5.0% 2.5% 5.0% 2.5% 5.0%
control compost AC biochar vermicompost
Variants

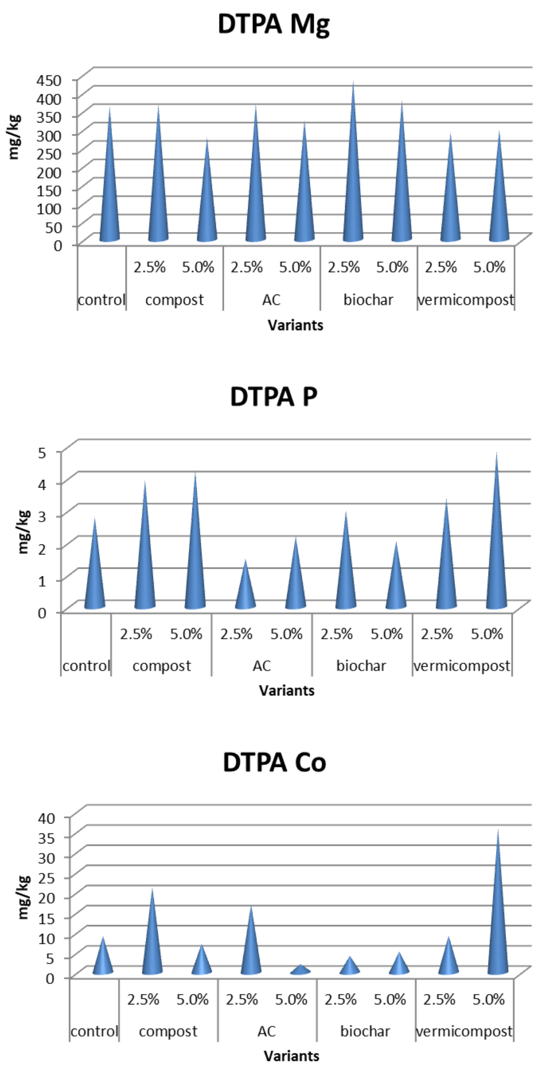


Figure. 3. Influence of organic additives on mobile forms of elements in soils

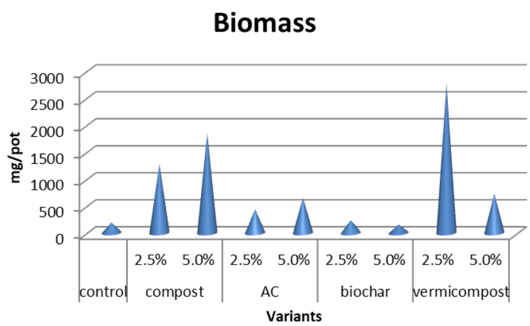
4.4. Assessment of the impact of organic amendments on plant development

4.4.1. Effect of soil amendments on plant biomass

Biometric studies were carried out to study the effect of organic amendments on plant development. Plant biomass in fresh and dry states was determined by weight at harvest. Figure 4 shows the impact of organic amendments on plant development. There were no significant differences in biomass production between the tested additives. Plant growth was significantly lower in the untreated soil, and plant biomass reached 195 mg. The application of organic additives had a pronounced beneficial effect on plant development and increased aboveground plant mass. When 5% activated charcoal and 5% biochar were added, the biomass increased to 665 mg and 766 mg, respectively. However, the most significant increase was with the application of compost, leading to an increase in plant biomass to 1281 mg (2.5% compost) and

1852 mg (5% compost). Increasing the compost rate from 2.5% to 5% resulted in a significant increase in plant biomass, consistent with Alvarez- Lopez et al. (2016).

Similar results were obtained by Thuringer (2020), who found that organic fertilisers led to a significant increase in O growth. chalcidica. This study confirmed previous findings that in serpentine soils characterised by low nutrient availability, the application of amendments had a positive effect on biomass production by the Ni-hyperaccumulating plant O. chalcidica (syn¼Alyssum murale) and Ni phytoextraction yield, respectively (Li et al. 2003; Bani et al. 2017, 2015b; Kidd et al. 2015; Alvarez- Lopez et al., 2016).



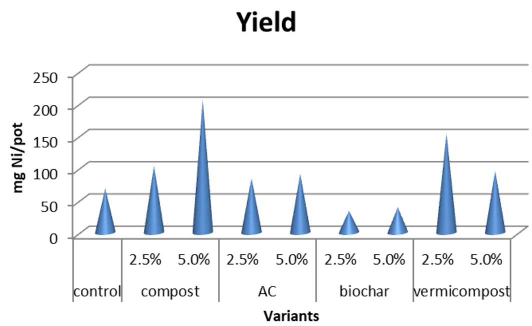


Figure.4. Effect of organic amendments on biomass and yield of Ni

4.4.2 Uptake of Ni, micro and macro elements by Alyssum murale (control)

Figure. 5 presents the results obtained for the Ni, micro and macro element contents in the above ground mass of the tested plant.

The Ni content in the aboveground mass of the control plants reached 3504.4 mg/kg, which significantly exceeded the threshold value of 1000 mg/kg set for Ni hyperaccumulation. The results confirm the findings of Bani (2009) and Broadhurst et al. (2004) that Ni is highly concentrated in epidermal cell vacuoles and that leaves contain the most Ni of all plant organs.

The amount of Ca taken up by the plants is high considering that these plants were grown on Ca-poor serpentine soils. The Ca content reaches 40004.2 mg/kg. The high Ca content in the leaves is the density of trichomes. Broadhurst et al. (2013) reported the exceptional Ca uptake in leaves of hyperaccumulator species (including the genus Alyssum). This is probably an adaptive ability of this genus (section Odontarrhenae) to Ca deficiency in serpentine soils. According to Proctor (1971), Ca is one of the elements influencing the reduction of heavy metal toxicity.

Bani et al. (2009) found that Ca and Ni can be antagonists, and the higher Ca content in Alyssum leaves can be explained by the lower Ni concentrations in A. murale leaves. The Ni: Ca ratio was lower than unity (0.088), and the Ni: Mg ratio was lower than unity (0.12).

Although Mg content is high in soils, Mg accumulates to a significantly lesser extent from Alyssum compared to Ni and Ca. The Mg content reaches 29412.7 mg/kg. A correlation between Ca and Ni uptake (0.82) and Mg and N uptake (0.73) was found in Alyssum leaves, similar to other authors. A correlation was found between the uptake amounts of Ca and Ni (0.82) and Mg and N (0.73) in Alyssum leaves, similar to other authors.

The Ca/Mg ratio in the aboveground mass was > 1. Plants from serpentine soils have the ability to maintain a ratio greater than one despite the low Ca content of the soil (and unfavourable Ca/Mg ratio for plant growth). Plants probably have very efficient Ca uptake systems or the ability to exclude Mg despite high soil Mg concentrations. Ni hyperaccumulators have been found to uptake large amounts of Ca from soils, and due to the repeated collection and removal of biomass during phytoremoval, agrotechniques such as Ca fertilisation are necessary to maintain soil fertility to appropriate levels (Chaney et al., 2008; Kukier et al., 2004). Some authors recommend adding gypsum to serpentine soils to overcome this limitation (Cabello-Conejo et al. 2013), but organic amendments (such as the compost and vermicompost used here) could also be used for this purpose.

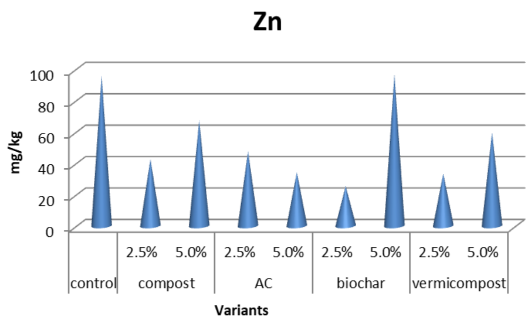
The P content reached 1720.6 mg/kg and 22127.0 mg/kg for K. Potassium is a key plant nutrient whose most important role is the maintenance of plant water balance (osmoregulation). Although K is low in serpentine soils, it accumulates in the aboveground mass of hyperaccumulator plants.

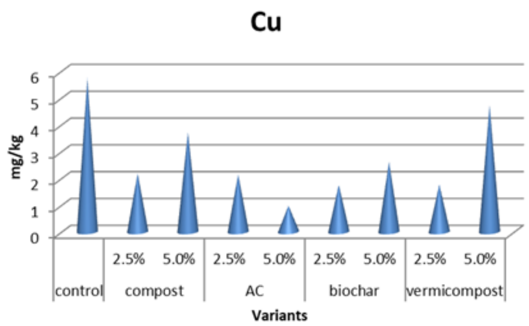
The Fe content reaches up to 1422.7 mg/kg in the plants tested. Fe is also essential for many plant functions as it is a constituent of some enzymes and proteins and plays a role in energy transfer in the plant. Similar values for the Fe content in the aboveground mass of O. Chalcidica have also been found by other authors (Bani et al., 2014; Broadhurst and Chaney, 2016; Xhaferri et al., 2018).

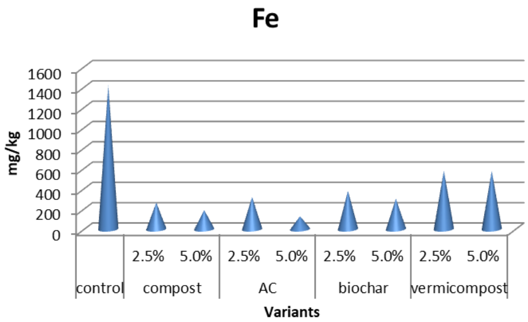
The content of Mn reached up to 672.6 mg/kg, Co up to 160.1 mg/kg, Zn up to 95.7 mg/kg and Cu up to 5.7 mg/kg.

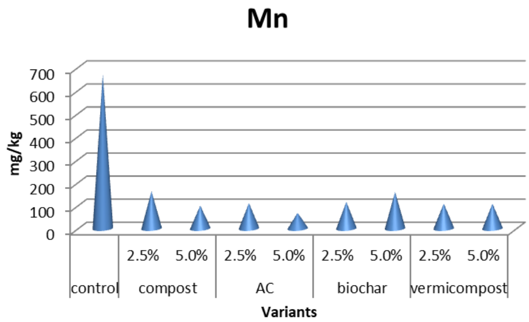
The low Cr content in A murale confirms the findings of Brooks (1987) that serpentine plants, without exception, contain only trace amounts of Cr. Despite high total soil Cr, the amount of Cr assimilated by A. murale is only 13.9 mg/kg. Plants from serpentine soils have generally contained <15 mg/kg Cr (Reeves, 1992).

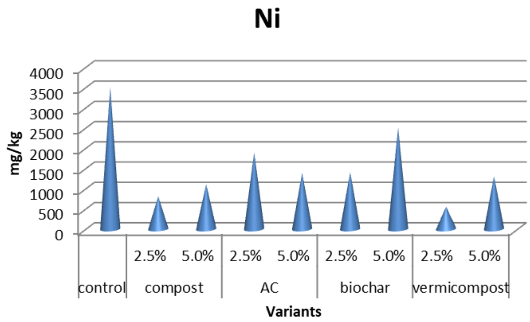
Ni uptake efficiency was calculated using the bioaccumulation factor (BAF=Cshoots/Csoils). BAF values indicate that Ni in plant tissue of O. chalcidica was up to 2.2 times higher than in soil. These values show the ability of O. chalcidica to accumulate Ni in its aboveground mass. Plants also assimilate significant amounts of P and Ca despite their low content in the soil. Our results confirm that the tested plant is a hyperaccumulator and can be used for Ni phytoextraction.

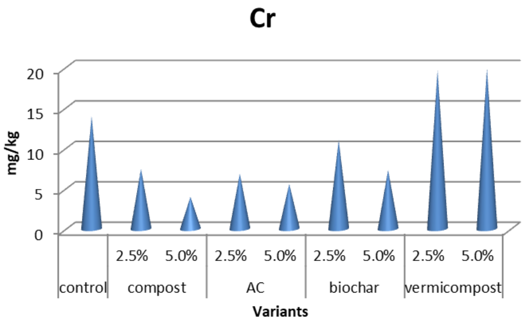


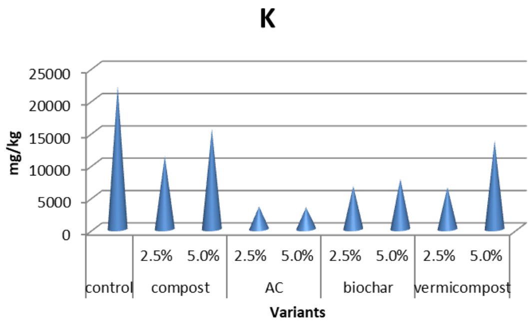


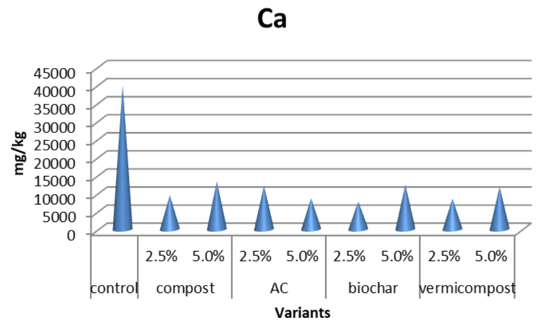

Fe
1600
1400
1200
1000
800
600
400
200
0
mg/kg
2.5% 5.0% 2.5% 5.0% 2.5% 5.0% 2.5% 5.0%
control compost AC biochar vermicompost
Variants

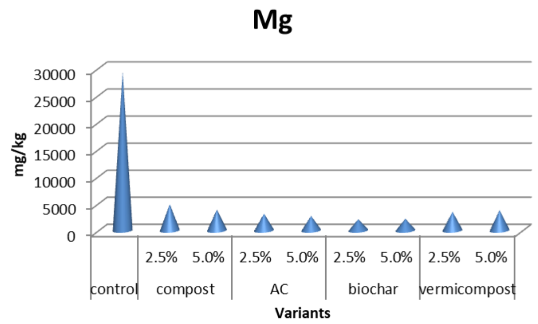

Mn
700
600
500
400
300
200
100
0
mg/kg
2.5% 5.0% 2.5% 5.0% 2.5% 5.0% 2.5% 5.0%
control compost AC biochar vermicompost
Variants

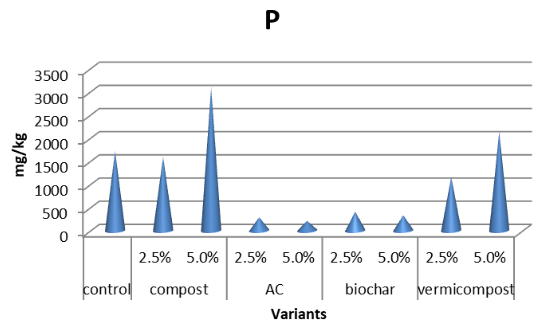

Ni
4000
3500
3000
2500
2000
1500
1000
500
0
mg/kg
2.5% 5.0% 2.5% 5.0% 2.5% 5.0% 2.5% 5.0%
control compost AC biochar vermicompost
Variants

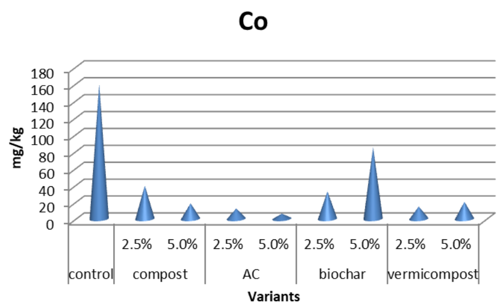

Cr
20
15
10
5
0
mg/kg
2.5% 5.0% 2.5% 5.0% 2.5% 5.0% 2.5% 5.0%
control compost AC biochar vermicompost
Variants

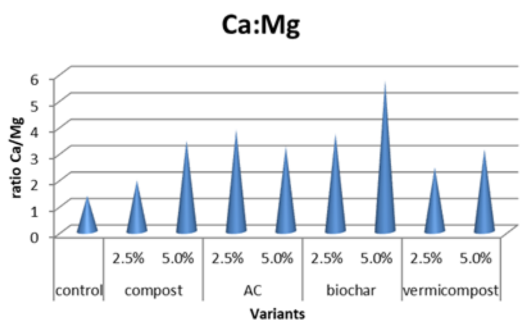

K
25000
20000
15000
10000
5000
0
mg/kg
2.5% 5.0% 2.5% 5.0% 2.5% 5.0% 2.5% 5.0%
control compost AC biochar vermicompost
Variants


Ca
mg/kg
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
2.5% 5.0% 2.5% 5.0% 2.5% 5.0% 2.5% 5.0%
control compost AC biochar vermicompost
Variants


Mg
mg/kg
30000
25000
20000
15000
10000
5000
0
2.5% 5.0% 2.5% 5.0% 2.5% 5.0% 2.5% 5.0%
control compost AC biochar vermicompost
Variants


P
mg/kg
3500
3000
2500
2000
1500
1000
500
0
2.5% 5.0% 2.5% 5.0% 2.5% 5.0% 2.5% 5.0%
control compost AC biochar vermicompost
Variants


Co
mg/kg
180
160
140
120
100
80
60
40
20
0
2.5% 5.0% 2.5% 5.0% 2.5% 5.0% 2.5% 5.0%
control compost AC biochar vermicompost
Variants


Ca:Mg
ratio Ca/Mg
6
5
4
3
2
1
0
2.5% 5.0% 2.5% 5.0% 2.5% 5.0% 2.5% 5.0%
control compost AC biochar vermicompost
Variants

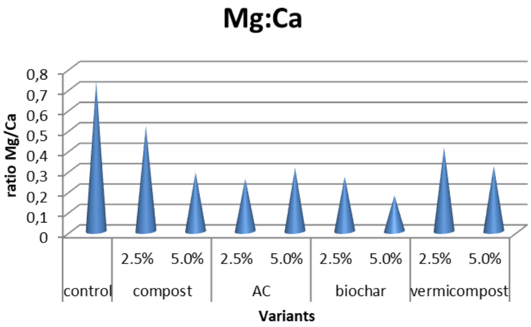


Figure.5 Influence of organic additives on the content of nickel, mirco and macroelements

4.4.3. Influence of organic amendments on the accumulation of Ni, micro and macro elements by Alyssum murale

● Influence of organic amendments on the accumulation of Ni in aboveground mass

The application of organic amendments significantly influenced Ni uptake by the plants tested. Figure. 5 presents the results obtained for the influence of the applied additives on the Ni content in the aboveground mass of O. chalcidica. The Ni content of the tested plants was higher in the control compared to the variants with the application of additives. Application of organic additives resulted in lower Ni content in the plants, influenced by the type of additive and dose. The application of a higher dose of the additives resulted in a smaller decrease in the Ni content of the plants. In plants where compost was applied to the soil, the Ni content ranged from 810.0 mg/kg (2.5% additive) to 1112.3 mg/kg (5% additive). Similar results were obtained when vermicompost was applied. Slightly higher values were found for the activated carbon and biochar input treatments, which reached 1901.3 mg/kg (5% activated carbon) and 2505.0 mg/kg (5% biochar). Our results confirm the results of Bani et al. (2007), which suggest that the cause is the dilution process in a closed system. Similar results were also obtained by Alvarez-Lopez et al. (2016), who found that the addition of compost significantly reduced the amount of Ni compared to plants growing in untreated soils. The authors found that increasing the compost rate (from 5% to 10%) was accompanied by a further decrease in the Ni concentration in the aboveground mass. A correlation was found between Ni content in the aboveground mass and NiDTPA.

● Effect of organic amendments on the accumulation of macro and trace elements in the aboveground mass of Alyssum murale

The uptake of micro and macro elements by the test plant was affected by the applied organic amendments. In most cases, there was a tendency for their content to decrease after using the organic additives (Figure. 5).

The application of organic additives leads to a decrease in the content of the trace elements zinc, Cu, Fe and Mn in the plants. The trend is similar for the macroelements K, Ca and Mg. According to Alvarez-Lopez et al. (2016), compost application leads to a decrease in Mg concentration, less pronounced in plants grown at the highest compost rate of 10%. The authors also found that compost application led to a significant reduction in the Fe concentration in the aboveground mass. According to the authors, the Fe and Mg contents of the aboveground mass decreased (in both composted and NPK-treated soils), but neither of the two elements reached deficiency levels (50- 150 mg/kg for Fe, 1.5-3.5 g/kg for Mg, according to Marschner, 1995), which is confirmed by our results. Correlations were found between plant uptake of Mg and Fe (0.92), Mg and Mn (0.99) and Mg and cobalt (0.86).

A probable reason for the lower elemental content in the aboveground mass of the variants with the application of the organic additives compared to the control was the increase in pH. After application of the organic amendments (6.4-7.6), the high soil pH is responsible for the formation of poorly soluble oxides of Fe, Mn, and Cr, resulting in their unavailability to plants (Babalonas et al., 1984).

According to Alvarez-Lopez et al. (2016), plants grown in soils enriched with compost also showed higher K concentration in aboveground mass than plants grown in untreated soil, which was not confirmed in the present study.

Regarding P, an increase in its content in the plants was found in the variants with the addition of 5% compost and 5% vermicompost. The addition of activated carbon and biochar resulted in its significant reduction. Similar abstracts were obtained by Alvarez-Lopez et al. (2016), who found an increase in aboveground P content when compost was added to the soil.

The application of vermicompost led to an increase in chromium content in plants. The opposite trend was observed for the other amendments.

According to Alvarez-Lopez et al. (2016), the Ca content of the aboveground mass decreased in plants grown in NPK-treated compared to untreated soils, while the addition of compost at 5% and 10% resulted in a significant increase in Ca content, which was not confirmed by our results.

The application of organic amendments also affected Ca: Mg and Mg: Ca ratios. The Ca: Mg ratio in both the control and all treatments with organic supplementation was greater than 1. The addition of compost increased the ratio from 1.36 in the control to 3.45, activated carbon to 3.22, biochar to 5.72 and vermicompost to 3.11.

The Mg: Ca ratio decreased from 0.74 in control to 0.29 when 5% compost was applied, to 0.31 in the 5% activated carbon option, to 0.17 when biochar was applied and to 0.32 when 5% vermicompost was applied. A correlation was found between Ca and Mg uptake by plants.

The Ca/Mg ratio from the soil was shown to be a relatively important factor in the growth and uptake of Ni by hyperaccumulators. Ghasemi and Ghaderian (2009) studied the Ca/Mg ratio effect on hyperaccumulator A. inflatum and found that the plant has higher tolerance at a low Ca/Mg ratio. The same authors found an increase of Ni in plants with an increasing Ca/Mg ratio in hydroponic solutions. We obtained similar results, which showed that the application of organic additives in the amount of 5% leads to an increase in the Ca/Mg ratio in the soil in which the uptake of Ni by plants increases. Opposite results were obtained by Thuringer, 2020 who found a decrease in Ni concentration in the aboveground mass with increasing Ca/Mg ratio in compost-enriched soil.

Similarly, Brooks et al. (1981) showed that high soil Ca concentrations resulted in lower uptake of Ni by A. serpyllifolium. For this reason, when applying organic ame ndments (compost, vermicompost, etc.) it is important to regulate the Ca/Mg ratio in the soil. To obtain an optimal balance between plant growth and Ni accumulation in the aboveground mass for optimum phytoremoval. The addition of compost and vermicompost can increase the organic matter content and fertility of serpentine soils. Still, it is also important to optimise the amount of amendments to achieve optimum plant growth and metal extraction. Field studies also need to be conducted to evaluate the effectiveness and potential benefits of compost and vermicompost application to the soil for Ni phytoextraction.

- Yield

Ni yield was also calculated. Serpentine soils usually have unfavourable conditions for plant growth, e.g. deficiencies of essential nutrients (such as N, P and K) and organic matter. Maximising the yield of hyperaccumulator crops is essential in plant production. This study shows that the application of organic amendments can improve soil fertility and hence plant growth on serpentine soils. The application of organic additives has a significant effect on the yield of Ni from plants, as the type of additive and the dose have an influence.

On the one hand, a decrease in the Ni content of the aboveground plant mass was found when they were applied to the soil, but on the other hand, a significant increase in plant biomass was recorded. Phytoextracted Ni increased after adding the organic additives due to the stimulation of plant growth. The results obtained after calculating the yield showed that the organic additives had a positive effect on the yield. In the variant with a 5% compost application, the Ni yield was three times higher than the control. Similar results were obtained with a 2.5% vermicompost application. Biochar application resulted in a twofold decrease in Ni yield, while activated carbon application resulted in a 1.2-1.3-fold increase (Figure.4).

Similar results were obtained by Bani (2007), who found that when Allyssum murale was grown for 6 months, the phytoextraction yield of Ni was 84.8 mg Ni in untreated plants, 399 mg Ni in treatments with 260N:105P:260K kg/ha pig manure; 265 mg Ni in treatments with 260N:390P:260K kg/ha chicken manure; and 152 mg Ni in treatments with 50:50:50 15 kg/ha N, P, K. Significant increases in yield and were also found by (Thuringer, 2020) in NPK- and compost-treated soils which suggested that the addition of compost had a higher growth-promoting effect than inorganic fertilisation. These results support previous studies that also showed stimulation of biomass production of Alyssum species following the addition of inorganic fertilisers (Cabello-Conejo et al., 2014b; Chaney et al., 2008; Robinson et al., 1997). Robinson et al. (1997) and Kidd and Monterroso (2005) found a reduction in Ni in the aboveg round mass of hyperaccumulator A. bertolonii and A. pintodasilvae after fertilisation in parallel with increased biomass production. However, these authors noted that Ni yield was increased due to increased plant biomass production.

The application of organic amendments (compost, vermicompost, activated carbon, and biochar) positively influenced the improvement of serpentine soil quality. All tested organic amendments are a source of essential nutrients (which are deficient in serpentine soils) to improve soil structure, porosity, and water holding capacity, which may explain the positive effect of amendments on plant yields. The addition of compost, vermicompost, biochar and activated carbon improves soil physicochemical properties such as nutrient content, CEC, organic matter content and improves the Ca/Mg ratio in the soil.

Phytoextracted Ni increases after adding organic amendments due to the stimulation of plant growth. In the treatments with 5% compost and 2.5% vermicompost application, plant Ni yield was three times higher than the control. However, field studies need to be conducted to evaluate the effectiveness and potential benefits of compost and vermicompost application to soil on Ni yield.

5. CONCLUSIONS

On the basis of the obtained results, the following more important conclusions can be drawn:

1. The application of organic additives to the soil influences the physicochemical properties and leads to an increase in organic matter and the content of macroelements (P, K, Ca and Mg) and trace elements (Fe, Mn, Zn) in the soil. This increase depends on the type and composition of the organic additive.

2. Organic additives reduce the amount of DTPA-extracted mobile forms of Ni, which is proportional to the amount of additive applied.

3. The amount of biomass is affected by the type and amount of organic additives used. Plant biomass increased significantly, with a clear correlation between the amount of additive applied and the increase in biomass.

4. Applying organic additives to the soil affects the uptake of Ni, micro and macro elements. There was a distinct trend of decreasing Ni content in the test plant after applying all four additives in the soil.

5. Organic additives positively affect yield as influenced by additive type and dose. Ni yield was three times higher in the treatments with 5% compost and 2.5% vermicompost application. Biochar application resulted in a twofold decrease in Ni yield, while activated carbon application resulted in a 1.2-1.3-fold increase.

6. It is necessary to optimise the amount of additions and adjust the Ca/Mg ratio in the soil to obtain an optimal balance between plant growth and Ni accumulation in the above-ground mass for optimum phyto yield. Field studies also need to be conducted to evaluate the effectiveness and potential benefits of compost and vermicompost application to the soil for Ni phytoharvesting.

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