

## Effects of Vanadium and Nickel on Morphological Characteristics and on Vanadium and Nickel Uptake by Shoots of Mojito (*Mentha × villosa*) and Lavender (*Lavandula angustifolia*)

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### Abstract

Vanadium and Nickel may enter the human food chain through medicinal and culinary plants which in great doses are toxic to human, so it is important to determine their potential toxicity and health risk. Therefore, the objective of this work was to study the effects of Vanadium and Nickel on morphological characteristics and on Vanadium and Nickel uptake by shoots of mojito (*Mentha × villosa*) and lavender (*Lavandula angustifolia*). A completely randomized block design with five Vanadium treatments (0, 5, 10, 20, 40 mg V L<sup>-1</sup>) and five replications per treatment and another one with five Nickel treatments (0, 5, 10, 20 and 40 mg Ni L<sup>-1</sup>) and five replications per treatment for mojito and lavender were conducted in pots. No visible toxic or inhibitory symptoms were observed on the plants due to the increasing amounts of Vanadium or Nickel. Shoot dry matter and root dry matter of mojito and lavender decreased with increasing Vanadium rates. Vanadium uptake by shoots of mojito and lavender increased linearly with increasing Vanadium rates. Nickel uptake by shoots of lavender increased linearly with increasing rates of Nickel. Mojito is a Nickel accumulator.

**Keywords:** culinary herbs; heavy metals accumulators; medicinal herbs

**Abbreviations:** SDM - shoot dry matter; STN - stem number; STL - stem length; RDM - root dry matter; RL - root length

### Introduction

Vanadium (V) is a transition metal and is widely distributed in the earth's crust with an average concentration of 110 mg kg<sup>-1</sup> (Peterson and Girling, 1981). The concentration of vanadium in soil depends upon the parent material and on the industrial pollution (WHO, 1987). The fate of vanadium in soils depends on iron and aluminum oxides and hydroxides that determine vanadium mobility in soils and waters (Naeem *et al.*, 2007). The evidence that V is essential for the growth of higher plants is, however, not yet conclusively demonstrated. It does not meet the criteria of essentiality which set by Arnon and Wessel (1953). Toxicity of vanadium to plants has mainly been studied in nutrient solutions and starts from between

1 mg V L<sup>-1</sup> and 5 mg V L<sup>-1</sup> for the most sensitive species (Kaplan *et al.*, 1990). Vanadium is toxic to humans at high V concentrations and can cause irritation of the respiratory tract, although it has not been possible to determine the level of exposure that provokes such effects (Costigan *et al.*, 2001). Several medicinal plants manifest a tendency to take up higher amounts of heavy metals than other plants. Mojito Mint (*Mentha × villosa*) is a species of perennial herbs in the family Lamiaceae. It is commonly grown for its edible qualities. Often used as a domestic herbal remedy, being valued especially for its antiseptic properties and its beneficial effect on the digestion (Martins *et al.*, 2007). Lavender (*Lavandula angustifolia*) is a flowering plant in the family Lamiaceae, native to the Mediterranean areas. It is a strongly aromatic shrub growing as high as 1 to 2 m tall.

The leaves are evergreen and the flowers are pinkish-L, produced on spikes 2-8 cm long at the top of slender (Erland and Mahmoud, 2016).

Nickel (Ni) occurs abundantly in igneous rocks as a free metal or as a complex with iron. It stands at twenty-second position amongst most abundant elements in the earth crust (Sunderman and Oskarsson, 1991). Additionally, anthropogenic activities release Ni into the soil through various sources such as smelting, burning of fossil fuel, vehicle emissions, disposal of house hold, municipal and industrial wastes, metal mining, fertilizer application, and organic manures (Alloway, 2013). During the last decades, Ni has become a serious concern as its concentration has reached up to 26,000 mg kg<sup>-1</sup> in polluted soils (Conelli and Renella, 2013) and 0.2 mg L<sup>-1</sup> in polluted surface waters (Zwolsman and Van Bokhoven, 2007). Ni in adequate quantities has vital roles in a wide range of physiological processes, starting from seed germination to the productivity. Moreover, plants cannot complete their life cycle without adequate supply of this metal. Therefore, Ni belongs to the list of essential micronutrients. Besides this, at elevated level it alters all the metabolic activities of the plant such as water relation and mineral nutrition, causes enzyme inhibition, disrupts stomatal functioning, photosynthetic electron transport and degrades chlorophyll molecules, consequently minimizes the photosynthetic rate, and biological yield of plants (Yusuf *et al.*, 2011). Excessive Ni levels in the soil can result in toxicity to plants (Amari *et al.*, 2014). The common indicators of Ni phytotoxicity to plants include inhibition of germination, leaf spotting, chlorosis, abnormal flower shape, reduced growth of roots and shoots, deformation of plant parts, poor branching, and decreased yield. The toxicity of Ni in plants has become a world-wide problem threatening sustainable agriculture as well. The critical toxicity level of Ni is more than 10 mg kg<sup>-1</sup> DW in sensitive species (Kozlow, 2005), 50 mg kg<sup>-1</sup> DW in moderately tolerant species (Bollard, 1983; Asher, 1991) and 1,000 mg kg<sup>-1</sup> DM in Ni hyper accumulator plants such as Alyssum and Thalspi species (Kupper *et al.*, 2001; Pollard *et al.*, 2002). The impact of Ni toxicity on the physiology of plants depends on the type of plant species, growth stage, cultivation conditions, Ni concentration and exposure time (Kabata-Pendias, 2011) in the soil. In the absence of Ni, plants develop specific deficiency symptoms, like leaf tip burn due to excessive accumulation of urea whereas its excess has been reported to cause leaf necrosis and chlorosis of plants (Marschner, 2002; Seregin and Kozhevnikova, 2006). It is evident from available literature that Ni affects plant growth at cellular, organ and the organism level (Bishnoi *et al.*, 1993). Nickel has been tested for a small number of plants, thus the information available on this topic should be valuable. More information on Ni levels in plant tissues that are consumed by mammals, and its effect on plant growth, is required. Several medicinal and culinary plants manifest a tendency to take up higher amounts of heavy metals than other useful plants. Mojito (*Mentha x villosa*) and Lavender (*Lavandula angustifolia*) are medicinal and culinary herbs and up till now we have little information on the response to increasing additions of V or Ni in the soil. Consequently, the objectives of this research were to examine the effect of V and Ni on morphological characteristics (stem number (STN), stem length (STL), root length (RL)), shoot (SDM) and root dry matter

(RDM)), and on V and Ni uptake by shoots of mojito and lavender.

## Materials and Methods

### Experimental conduction and design

A completely randomized block design with five V treatments (0, 5, 10, 20, 40 mg V L<sup>-1</sup>) and five replications per treatment for mojito and lavender, and another one with five Ni treatments (0, 5, 10, 20 and 40 mg Ni L<sup>-1</sup>) and five replications per treatment for mojito and lavender were conducted in pot experiments (50 pots for mojito and 50 pots for lavender = 100 pots totally). The plants grown on pots filled with peat and perlite medium (1:1 v/v) with pH 6.9. Vanadium was applied as NH<sub>4</sub>VO<sub>3</sub> and Ni as NiCl<sub>2</sub>·6H<sub>2</sub>O. Vanadium and Ni were added two times per week with 25 ml of each treatment per pot for five weeks (total 250 ml per pot of each treatment for the whole cultivation period, i.e. 14.3 mg V, 28.7 mg V, 57.4 mg V and 114.8 mg V for 5 mg V L<sup>-1</sup>, 10 mg V L<sup>-1</sup>, 20 mg V L<sup>-1</sup> and 40 mg V L<sup>-1</sup>, respectively and 25.3 mg Ni, 50.6 mg Ni, 101.2 mg Ni and 202.5 mg Ni for 5 mg Ni L<sup>-1</sup>, 10 mg Ni L<sup>-1</sup>, 20 mg Ni L<sup>-1</sup> and 40 mg Ni L<sup>-1</sup>, respectively. Fertilization of the pots was performed approximately every two weeks, using a commercial fertilizer (Nutrileaf-60) with 2 mg N, 2 mg P<sub>2</sub>O<sub>5</sub>, and 2 mg K<sub>2</sub>O for each pot (the content of V and Ni in the fertilizer was negligible).

### Plant analysis

At the end of the experiment, leaves, stems and roots were harvested. STN per pot as well as SL, RL per plant were measured. Then leaves stems and roots were oven-dried at 50 °C to constant weight, weighted and then grounded in a stainless steel Wiley mill and passed through a 150 µm plastic sieve, 0.5 g of plant parts smaller than 150 µm in diameter from each pot were placed in beakers and ashed at 450 °C. The residue was dissolved in 5 ml of 6N HCl. The clear solutions were analyzed by ICP-OES (Thermo Scientific iCAP 6000) for V and Ni.

### Soil analysis

At the end of each experiment samples of air-dried soil from each plant species and each pot were collected, passed through a 500 µm plastic sieve and analyzed for extractable V and Ni using the diethylene triamine penta acetic acid-triethanol amine (DTPA-TEA) method following procedure of Lindsay and Norvell (1978): 0.005M DTPA, 0.01M CaCl<sub>2</sub> and 0.1M TEA, was adjusted to pH 7.3 with 1N HCl. Ten grams of soil and 20 ml DTPA-TEA extracting solution were placed in polyethylene flasks covered with a plastic stopper and shaken by horizontal-circular movements at 240 oscillations per minute for 2 hours. The suspensions were filtered by gravity through Whatman no. 42 filter paper. The filtrates were analyzed for V and Ni by ICP-OES (Thermo Scientific iCAP 6000).

### Statistical analysis

ANOVA was used for statistical analysis using STATISTICA (2008). Where a significant difference was found, the Duncan's Multiple Range Test at the 5% level of probability was used to compare individual treatment means.

## Results and Discussion

### *Mojito and lavender as affected by Vanadium*

During the experiment no toxic symptoms or inhibitory effects on mojito and lavender due to increasing application rates of V were observed. The same results reported by Akoumianaki-Ioannidou *et al.* (2015) for pennyroyal and by Akoumianaki-Ioannidou *et al.* (2016) for sweet basil using the same amounts of added V. SDM and RDM of mojito decreased at rates above 10 and 5 mg V L<sup>-1</sup>, respectively (Table 1). SDM and RDM of lavender decreased significantly at rates above 20 and 5 mg V L<sup>-1</sup>, respectively (Table 2). STN and RL in mojito decreased at rates above 20 and 10 mg V L<sup>-1</sup>, respectively (Table 1). STN, STL and RL were not affected by increasing rates of V in lavender (Table 2). Vanadium uptake (Shoot Dry Mater x V concentration in shoots) by shoots of mojito and lavender

increased linearly with increasing V rates (Figs. 1 and 2). Singh (1971) detected an increase in dry matter when corn was subjected to doses of V from 0.05 to 0.25 mg L<sup>-1</sup>. Basiouny (1984) found that the dry matter of tomato plants increased when they were exposed to 0.2 mg V L<sup>-1</sup>. Vachirapatame *et al.* (2005) found that the growth of Chinese green mustard and tomato plants was retarded by nutrient solutions containing at least 40 mg V L<sup>-1</sup>. Akoumianaki-Ioannidou *et al.* (2015) for pennyroyal treated with the same V treatments as our own experiment reported that LDM and RDM increased with doses above 5 mg V L<sup>-1</sup>. Also Akoumianaki-Ioannidou *et al.* (2016) for sweet basil treated with the same V treatments as our own experiment, reported that LDM was not affected by V addition, in contrast RDM increased linearly with increasing V additions.

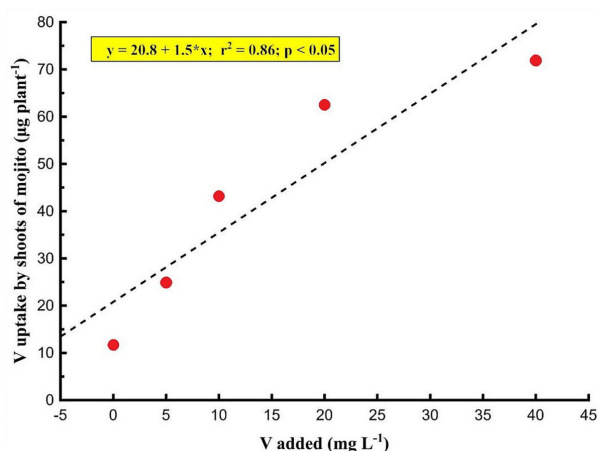


Fig. 1. Vanadium uptake by shoots of mojito as affected by Vanadium

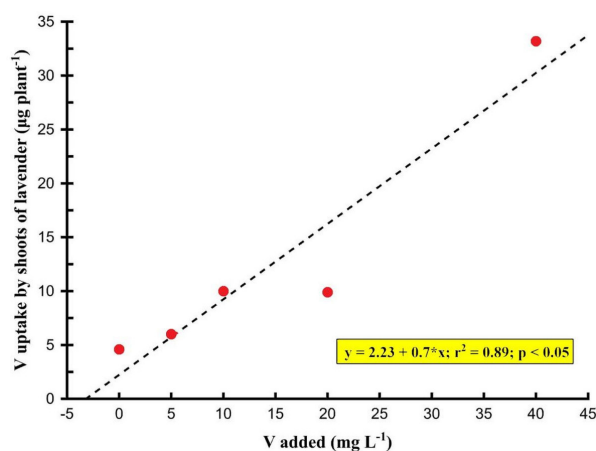


Fig. 2. Vanadium uptake by shoots of lavender as affected by Vanadium

Table 1. Effects of V treatments on morphological characteristics (shoot dry matter (SDM), stem number (STN), stem length (STL), root length (RL), and root dry matter (RDM)) of mojito, and on V concentration in soil

V added (mg L <sup>-1</sup> )	SDM (g)	STN	STL (cm)	RL (cm)	RDM (g)	V (soil) (µg g <sup>-1</sup> soil)
0	20.4 a	29.2 a	29.6	21.8 b	70.3 b	4.9
5	18.6 a	28.6 a	29.0	19.6 ab	56.5 b	4.8
10	17.6 ab	25.0 a	27.4	20.0 ab	32.5 a	5.3
20	14.0 b	23.8 a	27.4	13.8 a	33.9 a	5.1
40	13.9 b	17.8 b	24.6	14.2 a	25.9 a	4.9
F =	3.39			2.8	3.38	

Column means followed by the same letter are not significantly different, according to Duncan's multiple range test, at  $p \leq 0.05$ ; Column means without letters indicate no significance by Duncan's test at  $p \leq 0.05$

Table 2. Effects of V treatments on morphological characteristics (shoot dry matter (SDM), stem number (STN), stem length (STL), root length (RL), and root dry matter (RDM)) of lavender, and on V concentration in soil

V added (mg L <sup>-1</sup> )	SDM (g)	STN	STL (cm)	RL (cm)	RDM (g)	V (soil) (µg g <sup>-1</sup> soil)
0	11.8 b	39.0	16.4	13.1	9.6 b	8.6
5	10.6 ab	35.0	15.6	11.9	7.5 ab	8.9
10	10.4 ab	32.6	14.6	10.3	6.6 b	8.8
20	10.5 ab	31.5	13.0	11.9	5.4 b	8.8
40	8.7 b	30.1	14.8	7.3	5.4 b	8.7a
F =	2.61				2.6	

Column means followed by the same letter are not significantly different, according to Duncan's multiple range test, at  $p \leq 0.05$ ; Column means without letters indicate no significance by Duncan's test at  $p \leq 0.05$

*Mojito and lavender as affected by Nickel*

No visible toxic or inhibitory symptoms were observed on the plants due to the increasing rates of Ni during the experiment. SDM, STN, STL, RDM, and RL were not affected by Ni in the studied plants (Tables 3, 4). Ni uptake by lavender increased linearly with increasing rates of Ni (Fig. 3). Nickel uptake by mojito did not affected by Ni and is greater than the added Ni and approximately twenty times greater than that extracted with DTPA – TEA Ni from soil (Table 3). These results in combination with the fact that no toxic or inhibitory symptoms were observed in plants of mojito lead us to conclude that mojito is possibly a Ni accumulator, but more research is needed on this. Other researchers reported that exposure to 0.085-0.255 mM (5-15 ppm) Ni, for a week, developed chlorosis and necrosis along the veins in newly developed leaves of water spinach (Sun and Wu, 1998). Nickel at a concentration of 0.5 mM produced dark brown necrotic spots along the leaf margins

and decreased water potential and transpiration rate, resulting in the wilting of outer leaves (Panday and Sharma, 2002). Similarly, barley grown in 0.1 mM Ni for 14 days had foliar chlorosis and necrosis (Rahman *et al.*, 2005). Bashmakov *et al.* (2006) also observed a significant decrease in leaf area even at lower doses (50 and 0.1 mM) of Ni.

Vanadium and Ni extracted by DTPA-TEA from soil were not affected by V and Ni (Tables 1, 2, 3, 4) and did not correlated with V or Ni uptake by shoots in the studied plants indicating that this extractant should not be useful to predict V and Ni uptake by shoots of mojito or lavender. Moustakas *et al.* (2001), Moustakas *et al.* (2011), and Akoumianakis *et al.* (2008) reported that this extractant should be used for predicting Cd concentrations in lettuce, radish, cucumber, endive, rocket and pot-grown marigold plants.

Table 3. Effects of Ni treatments on morphological characteristics (shoot dry matter (SDM), stem number (STN), stem length (STL), root length (RL), and root dry matter (RDM)) of mojito, and on Ni concentration in soil

Ni added (mg L <sup>-1</sup> )	SDM (g)	STN	STL (cm)	RL (cm)	RDM (g)	Ni uptake (µg plant <sup>-1</sup> )	Ni (soil) (µg g <sup>-1</sup> soil)
0	24.5	23.9	30.4	16.2	38.7	169.2	8.7
5	24.9	27.2	25.2	14.6	35.4	213.0	8.8
10	23.8	23.6	22.0	13.4	38.6	211.9	8.9
20	21.1	25.2	23.2	14.2	43.3	236.1	8.8
40	21.6	20.8	20.4	12.8	52.4	246.7	8.8

Table 4. Effects of Ni treatments on morphological characteristics (shoot dry matter (SDM), stem number (STN), stem length (STL), root length (RL), and root dry matter (RDM)) of lavender, and on Ni concentration in soil

Ni added (mg L <sup>-1</sup> )	SDM (g)	STN	STL (cm)	RL (cm)	RDM (g)	Ni (soil) (µg g <sup>-1</sup> soil)
0	11.2	14.8	14.8	9.3	9.3	9.7
5	10.8	13.6	13.6	9.7	9.7	9.8
10	10.5	17.0	17.0	9.7	9.7	9.9
20	9.4	13.2	13.2	11.5	11.5	9.6
40	9.4	16.4	16.4	12.9	12.9	9.4

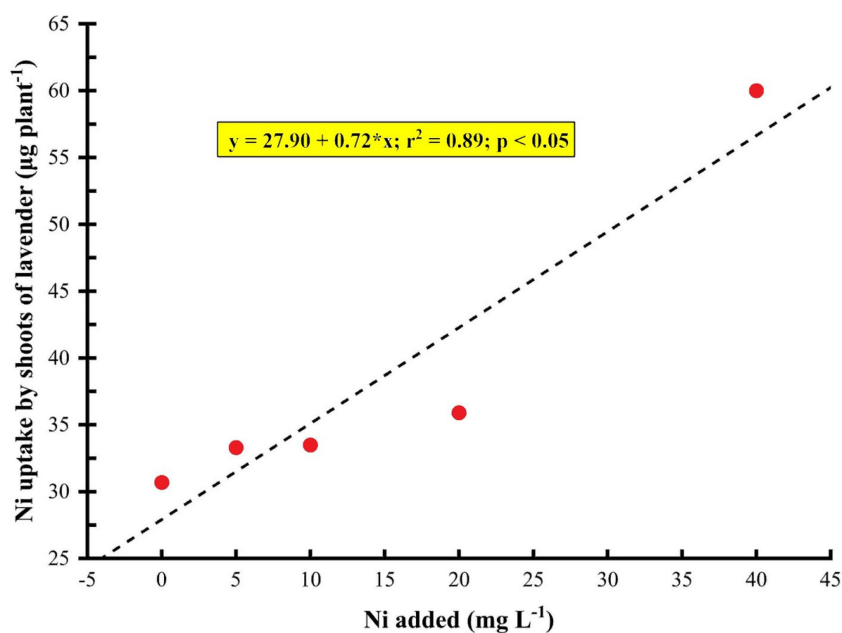


Fig. 3. Nickel uptake by shoots of lavender as affected by Nickel

## Conclusions

Mojito and lavender did not reveal toxic or inhibitory symptoms growth in soils contains up to 40 mg L<sup>-1</sup> V or Ni. Vanadium uptake by shoots of mojito and lavender increased linearly with increasing rates of V. Nickel uptake by shoots of lavender increased linearly with increasing rates of Ni. Morphological characteristics of Mojito and lavender were not affected by increasing rates of V or Ni. Mojito is a Ni accumulator. DTPA-TEA should not be used as extractant in order to predict V and Ni uptake by the above ground plant parts (shoots) of mojito and lavender.

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