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Agriculture (Poľnohospodárstvo), 63, 2017 (2): 52−66

Original paper DOI: 10.1515/agri-2017-0005

TRANSFER OF NICKEL FROM POLLUTED SOIL TO *PISUM SATIVUM* L. AND *RAPHANUS SATIVUS* L. UNDER COMPOSTED GREEN AMENDMENT AND NATIVE SOIL MICROBES

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NAFADY, N.A. – MAZEN, M.B. – AHMED, M.M.M. – MONSEF, O.A.: Transfer of nickel from polluted soil to *Pisum sativum* L. and *Raphanus sativus* L. under composted green amendment and native soil microbes. Agriculture (Poľnohospodárstvo), vol. *63*, 2017, no. 2, p. 52–66.

The effect of compost, inoculation with native soil microbes and their residual effects on bioavailability of nickel by peas (*Pisum sativum* L.) and radish (*Raphanus sativus* L.) grown on polluted soil were investigated in pot experiments. Plants were amendment with different compost levels (0, 0.2, 0.4, 0.6% of soil dry weight) and inoculated with different native soil microbes (4 fungal species, one bacterial species, 4 species of arbuscular mycorrhizal fungi) isolated from the polluted soil under study. Significant increases in the biomass of pea and radish plants were observed as a result of amendment application and their residual effects. The mycorrhizal dependency (MD) of pea plants was lower than of radish plants. The highest reductions of Ni levels in both plants were observed by the simultaneous applications of compost with microbes or mycorrhizal fungi to polluted soils. Soil pH increased significantly ($p < 0.05$) as a result of applying native microbes especially with arbuscular mycorrhizal fungi (AMF) alone or combined with compost. The DTPA extractability of soil Ni was significantly decreased with increasing soil pH ($p < 0.05$). The minimum transfer factor of Ni from polluted soil were 0.067 and 0.089 for pea and radish plants, respectively which were attained as a result of applying compost (0.6% of soil weight) inoculated with mycorrhizal fungi. From the results, we can conclude that the use of compost and native soil microbes as a soil remediate could be an effective strategy for soil remediation.

Key words: compost, native soil microbes, nickel, peas, radish

Nickel is an essential microelement for plants, animals, and humans, but toxic at high concentrations. Nickel adversely affects plant growth by altering different physiological and metabolic processes (Aziz *et al.* 2015). The toxic effects of nickel probably result primarily from its ability to replace other metal ions in enzymes and proteins or to bind to cellular compounds (Cempel & Nikel 2006). Excess Ni has been reported to cause leaf necrosis and chlorosis of plants (McIlveen & Negusanti 1994; Seregin & Kozhevnikova 2006). Nickel can accumulate in agricultural soils through the application of phosphate fertilisers, pesticides and other waste materials from industries like nickel-cadmium batteries, nickel electroplating, paints formulation, and vegetable fat production (Ramachandran & D'Souza 2013).

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Vegetables cultivated in soils polluted with toxic and heavy metals take up such metals and accumulate them in their edible and non-edible parts in quantities high enough to cause clinical problems both to animals and human beings. Toxic metals are known to have serious health implications, including carcinogenesis induced tumor promotion, and hence the growing consciousness about the health risks associated with environmental chemicals has brought a major shift in global concern towards prevention of heavy metal accumulation in soil, water and vegetables (Bhuiyan *et al.* 2011).

The risk of crop failure and economic losses, and decreasing human health risks from heavy metals can be reduce by using compost. Compost addition to soil alone can change the mobility and bioavailability of heavy metals in soil environment, as well as the toxic effects on plants and animals. These actions are attributed to various processes, including adsorption, complexation, precipitation, and redox reactions (Vaca-Paulin *et al.* 2006; Lagomarsino *et al.* 2011; Park *et al.* 2011; Huang *et al.* 2016).

Also, the use of microorganisms can minimize the bioavailability and biotoxicity of heavy metals (Gadd 2000; Lloyd & Lovely 2001). Another report cleared that the metal immobility in fungal-bacterial (FB) co-inoculation is governed by several mechanisms, including mass transfer of metals, biosorption, and precipitation (Bandara *et al.* 2015). Likewise, arbuscular mycorrhiza (AM) fungi can increase the tolerance of some plants to toxic metal contamination by developing the metal tolerance of the fungi themselves and binding the metals to polyphosphates within the fungal hyphae implicated (Barea *et al.* 2005; Morgan *et al.* 2005; Elgharably & Nivien 2013; Abd-Alla *et al.* 2016).

In the current investigation, peas followed by radish were grown in polluted soil with application of different levels of green compost and native soil microbes.

The objective of this work was studying the direct and residual effect of compost and native microbes and their combination on: (1) peas and radish plant growth; (2) nickel concentration in plant parts; (3) soil pH and Ni availability.

MATERIAL AND METHODS

Soil, compost and native microbes inocula

The experimental soil was collected from the polluted agriculture area near the superphosphate factory (27°N and 31°E), Assiut city, Egypt. Several samples (0–15 cm depth) were collected and bulked to give a composite sample. The soil texture was silt and classified as Typic Torrifluvents according to USDA Soil Taxonomy (2010). Soil had a pH of 6.50 (1:2 soil/water suspension). The electrical conductivity (EC), organic matter (OM) and total $CaCO_3$ were measured according to Jackson (1973) recorded 2.55 dS/m (1:2 soil water extract), 15.9, 15.2 g/kg soil, respectively. Total N (1.56 g/kg soil) was determined by Micro Kjeldahl method (Black 1965). Available P was 150 mg/kg soil (Olsen & Sommers 1982), total Ni and available Ni of soil samples were 56.75 and 0.501 mg/kg soil, respectively.

Compost heap was built alternately between corn residues, sheep manure, and peanut residues at a ratio of 70:20:10% respectively. During composting, materials were manually mixed every 15 days throughout the composting period for air circulation and temperature homogeneity. The moisture level of the heap was measured gravimetrically every week and appropriate amount of water was sprinkled onto the heap to increase the moisture content up to 60%. Compost heap reached to maturity after 4 months. Compost samples were dried at 70°C to constant weight ground. The value of pH was 8.6 determined in (1:10) [compost: water] suspension using glass electrode pH meter. The EC was 6.14 dS/m (compost water extract, 1:10). The organic matter (OM) content of the compost analysed by weight loss on ignition at 430°C for 24h was 540.9 g/kg, and total organic carbon (TOC) was 280.7 g/kg (calculated from OM) according to Navarro *et al.* (1993). The NPK contents were 20.9, 3.5 and 15.9 g/kg compost, respectively. Also, compost samples were digested using a nitric-perchloric acids mixture (HNO_3 + $HClO_4$) to determine total Ni (AOAC 1990) which recorded 2.77 mg/kg compost.

Native microbes of *Aspergillus niger, A. terreus*, *Penicillium funiculosum* and *Fusarium culmorum* were isolated from the polluted site under investigation on Potato Dextrose Agar (PDA). Inoculum potential of native isolated fungi was 10^4 cfu/g. Also, *Bacillus* sp. was isolated from the soil under study and the inoculum potential was 10^6 cfu/g. The compatible mixed culture was used as *A. niger, A. terreus, P. funiculosum* and *F. culmorum* and *Bacillus* sp. (Mohammad *et al.* 2011).

Four species of native mycorrhizal fungi were isolated from the polluted soil under study. The most dominant species was extracted by wet sieving and decanting technique (Gerdemann & Nicolson 1963). The species named *Acaulospora bireticulata* F.M. Rothwell & Trappe, *Gigaspora margarita* W.N. Becker & I.R. Hall, *Glomus lamellosum* Dalpé, Koske & Tews and *Funneliformis mosseae* (Nicol. & Gerd.) Walker & Schüßler. Mycorrhizal inoculum was propagated in a mixture of sand and bulk contaminated soil (1:1v/v) using *Zea mays* L. seedlings as host plants for 2 months. The inoculum consists of spores, hyphae and colonized root fragments of which 100 gram of AM fungi inoculum was placed below seeds of the tested plant (approximately contain 10 spores/g soil). The same volume of autoclaved inoculum was added below control seeds.

Experimental design

Factorial experiment in completely randomize block design was performed in greenhouse of Assiut Agricultural Research Station, Egypt. Plastic rectangular pots (35 cm height × 26 cm width × 26 cm length) were filled with 18 kg of polluted soil under study. Four levels of dried compost (0, 0.2, 0.4 and 0.6% of soil weight as CS0, CS1, CS2, CS3) were mixed with the polluted soil and inoculated with three native microbe treatments. FB (four fungal species + one bacterial species); AMF (arbuscular mycorrhizal fungi) and FB+AMF. Five seeds of peas (*Pisum sativum* L.) were planted as a seed vegetable in each pot. Ammonium nitrate (33.5% N) and potassium sulphate (50% K_2O) were used as a mineral fertiliser of all treatments. Three replicates were used for each treatment. Pea plants were harvested after 90 days from planting.

After harvesting peas, five seeds of radish (*Raphanus sativus* L.) were planted as a root vegetable in each pot to study the residual effect of the experimental treatments without adding any compost or inoculums, only mineral fertilisation was added. Plants were maintained at a soil water potential of field capacity. Plant samples were harvested after 30 days from planting.

Soil and plant analysis

Soils samples were collected after harvesting plants pea and radish, oven dried at 40°C for 48 h and passed through a 1 mm sieve. Soil pH was measured in 1:2 (soil: water) suspensions using a glass electrode according to Mclean (1982). Soil samples were digested with nitric and hydrochloric acid mixture according to Cottenie *et al.* (1982) for measurement of total nickel and determined by Inductively Coupled Plasma Spectrometry (Ultima 2 JY Plasma). The diethylene triamine penta acetic acid extracting (0.005M DTPA, 0.1 TEA, and 0.01 M $CaCl_2$, adjusted to pH 7.3) solution (Lindsay & Norvell 1978) was employed to extract Ni as a potential indicator of plant-available heavy metals from soils.

Plant samples were washed thoroughly with running tap water and rinsed three times with deionized water. The dry weights of pea (root, shoot, peels and seeds) and radish (root and shoot) plants were recorded after drying in a forced air oven at 65°C for 48h. Half gram of each plant sample was wet digested using a nitric-perchloric acids mixture (HNO_3 + $HClO_4$) according to the procedure of Tedesco *et al.* (1995) to determined total Ni in plant tissues.

To characterize quantitatively the transfer of an element from soil to plant, the soil-plant Partition Coefficient or Transfer Factor (TF) that expresses the ratio of contaminant concentration in plant parts to concentration in dry soil is used (Cui *et al.* 2004).

Determination of mycorrhizal colonization

One-cm long pieces of roots were used to investigate the mycorrhizal colonization of peas and radish. Fresh root samples were treated with 10% KOH and the AMF stained with trypan blue (0.1%) in lactophenol (Phillips & Hayman 1970). The percentage of root colonization was determined by the grid-intersect method (Giovannetti & Mosse 1980).

Quantification of mycorrhizal dependency

The mycorrhizal dependency (MD) of plant (shoot + root) growth was determined according to Plenchette *et al.* (1983) as follow:

$$\textit{Dependency of growth} = \frac{\mathrm{M-NM}}{\mathrm{M}} \times 100$$

where:
M and NM refer to the dry mass of mycorrhizal and non-mycorrhizal plants, respectively.

Statistical Analysis

Results were processed and analysed using SPSS statistical analysis package for Windows®. Data is reported as mean ± standard deviation of the mean unless otherwise stated. A p-value of < 0.05 was considered significant. Two-way analysis of variance was performed (ANOVA) on the pairs of variables likely to exhibit correlation.

RESULTS AND DISCUSSION

Bioavailablability of Ni and soil pH

Effect and residual effect of compost, microbes and arbuscular mycorrhizal fungi on DTPA extractability of soil Ni and pH of pea and radish soils after harvesting was detected in Table 1. Data showed that, the DTPA extractability of soil Ni was significantly decreased with increasing soil pH ($p < 0.001$). Soil pH increased significantly ($p < 0.05$) particularly when using AMF or FB + AMF only or combined with compost and their residual effects. Results showed that the pH of radish soil were always higher than the pH of pea soil. The soil availability of Ni under compost and inoculations and their residual effects was always lower than the control. Soil cultivated with pea and treated with AMF always had the lowest amounts of DTPA-extractable Ni with all discussed compost treatments 0.904 mg/kg dry soil by reduction 32.34% at the pH (7.12). On contrast,

T a b l e 1

Available Ni and pH of soils grown with peas and radish after harvesting

Compost	Inoculation	Peas		Radish	
		Available Ni [mg/kg]	pH (1:2)	Available Ni [mg/kg]	pH (1:2)
CS0	NU	1.342 ± 0.008^{k}	6.95 ± 0.04^{a}	1.140 ± 0.008^{k}	7.03 ± 0.05^{a}
	FB	1.316 ± 0.011^{ij}	6.97 ± 0.06^{a}	0.972 ± 0.006^{j}	7.05 ± 0.03^{ab}
	AMF	0.904 ± 0.012^{a}	7.12 ± 0.02^{gh}	0.840 ± 0.006^{d}	7.18 ± 0.02^{f}
	FB+AMF	1.184 ± 0.020^{g}	7.09 ± 0.01^{efgh}	0.698 ± 0.006^{a}	7.16 ± 0.01^{ef}
CS1	NU	1.336 ± 0.008^{jk}	7.00 ± 0.02^{abc}	0.928 ± 0.010^{i}	7.08 ± 0.03^{bc}
	FB	1.304 ± 0.008^{i}	6.98 ± 0.02^{ab}	0.894 ± 0.004^{h}	7.10 ± 0.02^{cd}
	AMF	0.927 ± 0.011^{b}	7.10 ± 0.01^{fgh}	0.692 ± 0.006^{a}	7.16 ± 0.03^{ef}
	FB+AMF	1.038 ± 0.014^{e}	7.10 ± 0.02^{fgh}	0.854 ± 0.007^{ef}	7.10 ± 0.02^{cd}
CS2	NU	1.194 ± 0.014^{g}	7.04 ± 0.05^{cde}	0.860 ± 0.006^{fg}	7.12 ± 0.02^{cde}
	FB	1.220 ± 0.012^{de}	7.03 ± 0.01^{bcd}	0.862 ± 0.004^{fg}	7.12 ± 0.01^{cde}
	AMF	0.938 ± 0.008^{b}	7.08 ± 0.03^{defg}	0.784 ± 0.004^{b}	7.12 ± 0.03^{cde}
	FB+AMF	1.026 ± 0.014^{de}	7.06 ± 0.03^{def}	0.844 ± 0.008^{de}	7.14 ± 0.01^{def}
CS3	NU	1.152 ± 0.008^{f}	7.05 ± 0.02^{cdef}	0.920 ± 0.006^{i}	7.09 ± 0.02^{bc}
	FB	1.194 ± 0.010^{g}	7.03 ± 0.03^{bcd}	0.884 ± 0.006^{h}	7.10 ± 0.02^{dc}
	AMF	0.962 ± 0.016^{c}	7.06 ± 0.01^{def}	0.868 ± 0.008^{g}	7.16 ± 0.01^{ef}
	FB+AMF	1.013 ± 0.007^{d}	7.14 ± 0.01^{h}	0.854 ± 0.007^{ef}	7.10 ± 0.02^{cd}
Two-way ANOVA					
CS		+++	+	+++	NS
IN		+++	+++	+++	+++
CS × IN		+++	+++	+++	+++

Mean ± SD (n=3). CS: compost amendments; NU: plants without inoculation; FB: plants inoculated with microbes; AMF: plants inoculated with arbuscular mycorrhizal fungi; FB+AMF: plants inoculated with microbes and arbuscular mycorrhizal fungi; CS: effect of compost; IN: effect of inoculation; CS × IN: the effect of interaction between compost and inoculation; One-way ANOVA was performed for amendment treatments; Means for each parameter with different letters are significantly different from each other ($p < 0.05$) according to the Duncan test; Two-way ANOVA was performed to determine the effect of compost, inoculation and their interaction on polluted soil. NS not significant; $^{+}p < 0.05$; $^{++}p < 0.01$; $^{+++}p < 0.001$.

the residual effect of FB plus AMF only and CS1 plus AMF treatments attained the lowest level of DTPA extractable Ni in radish soil record 0.698 and 0.692 mg/kg dry soil, respectively at soil pH (7.16) by reduction 38.77 and 39.30%, respectively. From results, the addition of compost and inoculation has reduced the availability of Ni. At high pH, metals tend to form insoluble metal mineral phosphates and carbonates, which are less bioavailable (Rensing & Maier 2003; Sandrin & Hoffman 2007). Also, mycorrhizal plants recorded the highest values in soil pH and lowest values of DTPA-extractable Ni. These results are in agreement with Bano & Ashfaq (2013) who reported that the immobilization of some heavy metals due to the mycorrhizal association may possibly be due to a slight increase in pH in the mycorrhizosphere. Many heavy metals are immobilized due to this change in pH under conditions of high concentrations of certain metals. The beneficial effects of mycorrhiza against plant's heavy metal uptake may be associated with heavy metal solubility caused by changes in soil pH.

Plant biomass

Data in Table 2 show the impact of different fertilisation strategies on peas growth and their residual effects on radish growth. The results indicated that using different compost levels and inoculation significantly (p < 0.05) increased the growth measurements of pea and radish plants. Data revealed that the highest values of roots and shoots dry

T a b l e 2

Effect and residual effect of compost, microbes and arbuscular mycorrhizal fungi on biomass of peas and radish

Compost	Inoculation	Peas				Radish	
		Dry weight [g/plant]		Peels dry weight [g/plant]	Seeds dry weight [g/pant]	Dry weight [g/plant]	
		Root	Shoot			Root	Shoot
CS0	NU	0.24 ± 0.01^{a}	11.70 ± 0.36^{a}	3.36 ± 0.37^{a}	3.97 ± 0.5^{a}	1.57 ± 0.07^{a}	3.90 ± 0.48^{a}
	FB	0.28 ± 0.02^{ab}	12.57 ± 0.21^{ab}	5.29 ± 0.19^{bcd}	6.75 ± 0.81^{def}	1.87 ± 0.30^{ab}	5.02 ± 0.47^{bc}
	AMF	0.32 ± 0.04^{bc}	12.73 ± 0.70^{ab}	6.15 ± 0.54^{def}	6.92 ± 1.08^{def}	2.55 ± 0.28^{d}	5.29 ± 0.20^{cd}
	FB+AMF	0.29 ± 0.02^{ab}	11.90 ± 0.79^{a}	6.50 ± 0.70^{efg}	5.64 ± 1.14^{bcd}	2.16 ± 0.33^{bcd}	5.67 ± 0.02^{defg}
CS1	NU	0.28 ± 0.03^{ab}	13.27 ± 1.96^{abc}	4.84 ± 1.08^{bc}	5.24 ± 0.80^{abc}	2.32 ± 0.23^{cd}	5.70 ± 0.48^{defg}
	FB	0.30 ± 0.05^{ab}	12.90 ± 0.45^{ab}	4.19 ± 0.16^{ab}	5.58 ± 0.27^{bcd}	1.59 ± 0.08^{a}	5.54 ± 0.27^{cdef}
	AMF	0.35 ± 0.03^{bc}	13.78 ± 0.74^{abcd}	6.76 ± 0.55^{fg}	8.12 ± 0.48^{fg}	2.17 ± 0.30^{bcd}	6.01 ± 0.25^{fg}
	FB+AMF	0.33 ± 0.04^{bc}	14.81 ± 0.54^{bcde}	4.32 ± 0.36^{ab}	7.11 ± 0.27^{ef}	1.97 ± 0.12^{abc}	5.34 ± 0.14^{cde}
CS2	NU	0.32 ± 0.04^{bc}	15.75 ± 1.85^{de}	6.29 ± 0.69^{def}	7.23 ± 0.18^{ef}	1.92 ± 0.03^{abc}	5.55 ± 0.21^{cdef}
	FB	0.34 ± 0.03^{bc}	13.79 ± 1.01^{abcd}	7.48 ± 0.46^{g}	8.81 ± 0.94^{g}	2.12 ± 0.14^{bc}	4.61 ± 0.19^{b}
	AMF	0.38 ± 0.03^{c}	16.82 ± 1.51^{e}	6.13 ± 0.69^{def}	7.42 ± 0.86^{ef}	2.55 ± 0.40^{d}	6.18 ± 0.32^{g}
	FB+AMF	0.30 ± 0.07^{ab}	14.39 ± 1.05^{bcd}	6.89 ± 0.65^{fg}	6.22 ± 1.02^{bcde}	2.11 ± 0.19^{bc}	5.78 ± 0.35^{defg}
CS3	NU	0.30 ± 0.03^{ab}	15.58 ± 2.07^{cde}	5.21 ± 0.45^{bcd}	6.47 ± 1.10^{cde}	1.84 ± 0.09^{ab}	5.33 ± 0.49^{cd}
	FB	0.29 ± 0.04^{ab}	13.64 ± 1.33^{abcd}	6.22 ± 0.80^{def}	5.48 ± 0.62^{bcd}	2.02 ± 0.21^{bc}	4.46 ± 0.13^{b}
	AMF	0.32 ± 0.04^{bc}	15.80 ± 2.17^{de}	5.48 ± 0.50^{cde}	6.71 ± 0.81^{def}	2.35 ± 0.25^{cd}	5.96 ± 0.32^{efg}
	FB+AM	0.28 ± 0.06^{ab}	13.34 ± 0.29^{ab}	4.73 ± 0.58^{bc}	4.90 ± 0.12^{ab}	2.21 ± 0.13^{bcd}	5.89 ± 0.40^{defg}
Two-way ANOVA							
CS		+	+++	+	++	NS	+++
IN		++	+	NS	++	+++	+++
CS × IN		NS	NS	NS	+	++	+++

Mean ± SD (n=9). One-way ANOVA was performed for amendment treatments; Means for each parameter with different letters are significantly different from each other (p < 0.05) according to the Duncan test; Two-way ANOVA was performed to determine the effect of compost, inoculation and their interaction on polluted soil. NS not significant; $^{+}p < 0.05$; $^{++}p < 0.01$; $^{+++}p < 0.001$.

weight of pea plants were attained in CS2 combined with AMF treatment and its residual effect on radish plants (58.33, 43.76, 62.42 and 58.46%, respectively). The dry weight of peels and seeds increased by 122.62 and 121.91%, respectively compared with the control (CS0 and NU) when 0.4% dry compost with FB (4 fungal species + one bacterial species) were applied. This might be explained by the effect of fertilisers enrichment with beneficial strains of bacteria and fungi on increasing fertilisation in crop production (Chen 2006) by enhancing the physiology of crop plants, stimulating their growth and yielding, as well as by increasing their resistance to environmental and biotic stresses (Corte *et al.* 2013). Also application of native mycorrhizal fungi and beneficial strains of bacteria and fungi incorporated ensures their better adaptation and survival in the prevailing environmental conditions, which is an extremely important factor for their long-term effects on plants (Regvar *et al.* 2003). Two-way ANOVA indicated that both "compost" and "inoculation" factors significantly affected ($p < 0.01$) all growth measurements of peas and radish expect root dry weight of radish and peels dry weight of peas. The interaction of "compost × inoculation" factor had a non-significant effect on all growth measurements of peas except seeds dry weight while all growth measurements of radish were significantly affected ($p < 0.01$).

Mycorrhizal colonization

Microscopic analysis confirmed that plants of non-inoculation treatments were not colonized by AMF. There were large differences between colonization patterns in pea roots (Figure 1A). The colonized roots were occupied by intercellular, intracellular hyphae, vesicles and arbuscules. Roots of pea plants treated with CS1+AMF and CS2+FB+AMF recorded the maximum values of hyphae, vesicles and arbuscular colonization. While, the highest proportions of mycorrhizal colonization structures in radish plants were recorded with the residual effect of CS3+FB+AMF treatment (Figure 1B).

Mycorrhizal dependency for peas and radish growth

Mycorrhizal dependency (MD) is defined as the degree to which a plant is dependent on the mycorrhiza to produce maximum growth or yield at a given soil fertility level. Results given in Figure 2A, B) clearly showed that the mycorrhizal dependency (MD) for radish plants was higher than for pea plants. MD was always higher with AMF inoculation than with FB+AMF inoculation in both plants. The highest MD value of pea plants obtained from CS2 treated plants inoculated with AMF (37.22%). While the lowest MD value (17.07%) was observed in plants treated with CS3 inoculated with FB+AMF. Also, the residual effect of AMF with CS2 realized the highest value (41.02%) of MD for dry weight of radish plants. While the minimum value of MD of radish dry weight was obtained in CS1+FB+AMF (29.02%). The increase in mycorrhizal dependency (MD) values to a certain level and then decline might return to the over fertilisation. High levels of fertilisation, often occurring in intensive agriculture, have a negative effect on root growth and root colonization by mycorrhizal fungi (Smith & Read 2008).

Nickel concentration in pea and radish plants

Data in Figure 3 (A-D) and 4 (A-B) show the effect of applied compost at different levels and inoculation and their residual effects on nickel concentration in roots, shoots, peels and seeds of peas and subsequent roots and shoots of radish plants. Compost at 0.6% (CS3) inoculated with fungal-bacterial inoculum plus AMF attained highly significant reduction for nickel content by 65.60 and 71.46% in roots and seeds of peas, respectively compared with the control treatment (CS0+NU). The lowest Ni values in shoots of peas were realized in CS3+AMF and CS3+FB+AMF with reduction of 60.21% compared with the control treatment. In pea peels, there were no significant differences between most treatments. Concerning to radish plants, the lowest level of Ni in shoots of radish was observed in CS3 and AMF treatment by a reduction of 38.46%. In radish roots, the residual effect of AMF, and FB+AMF treatments recorded the minimum content of Ni when existed individually or combined with compost at any level. Also, CS3 and CS3+FB treatments attained the lowest Ni concentration in plant parts. There were no significant differences between previous treatments. The effect of applied compost levels and inoculation on decreasing Ni concentration could be arranged in descending order of: CS+FB+AMF ≥ CS+MF > CS+FB > CS. In all treatments, the nickel concentration in peas and radish organs was higher than the

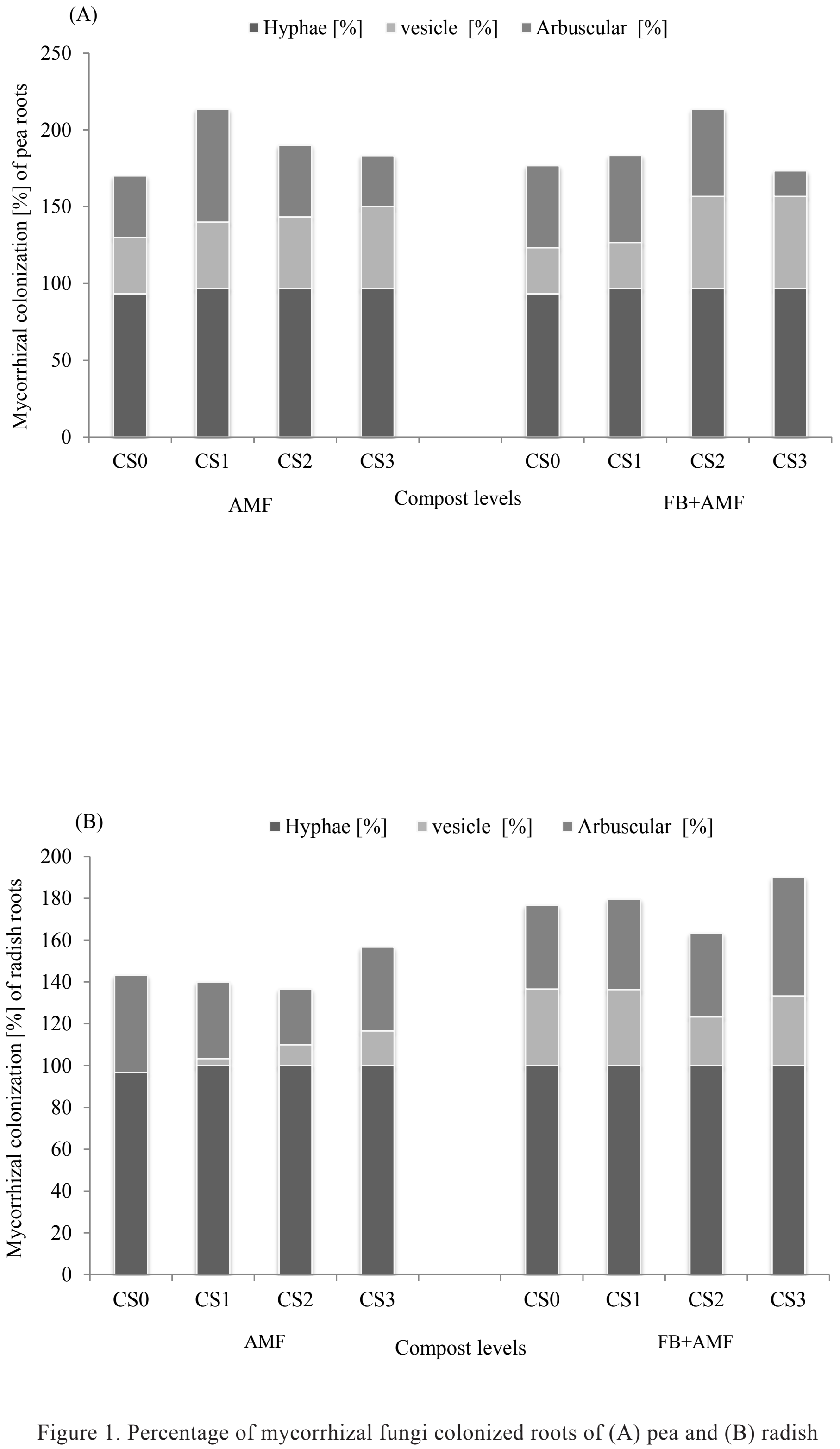


Figure 1. Percentage of mycorrhizal fungi colonized roots of (A) pea and (B) radish

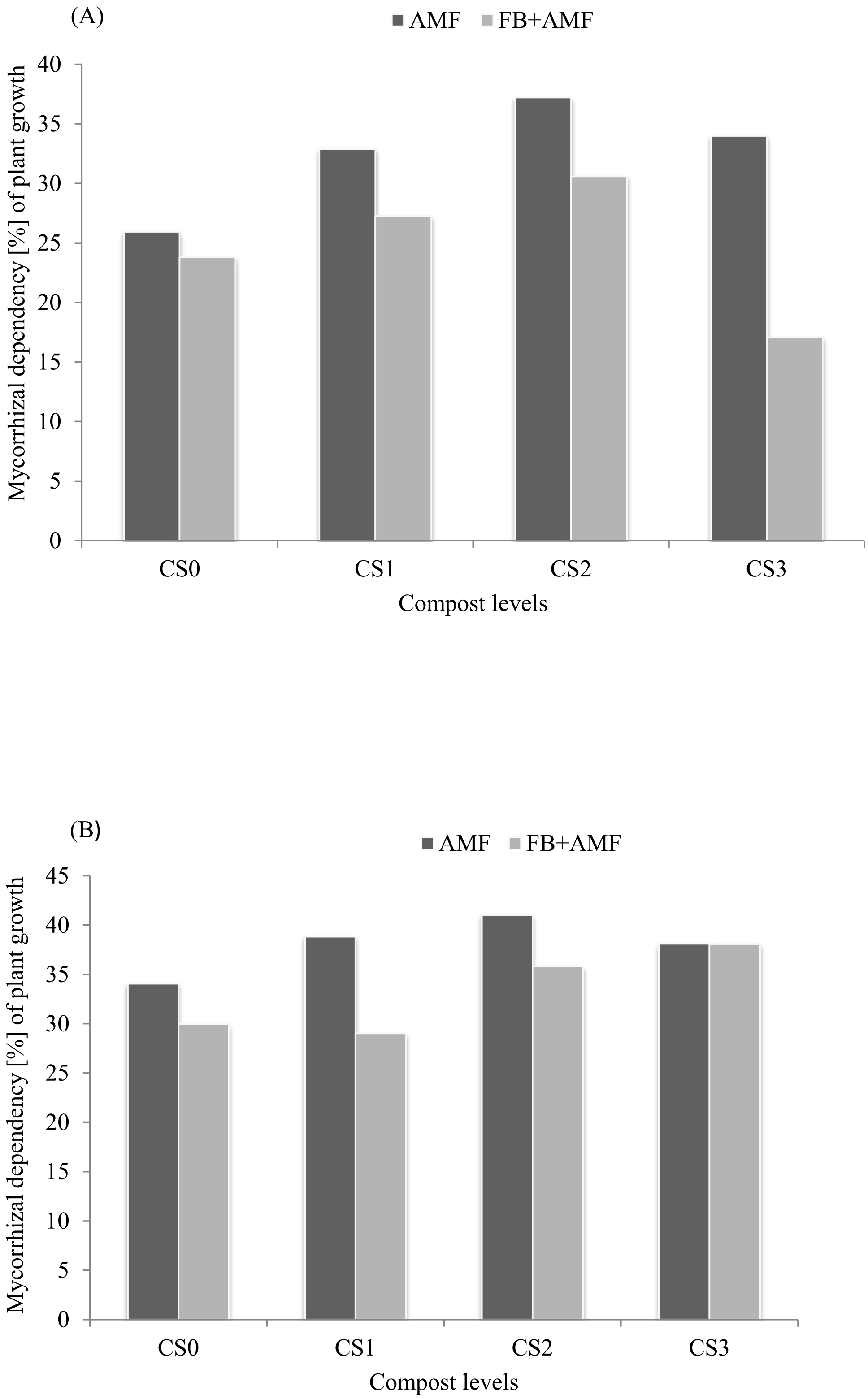
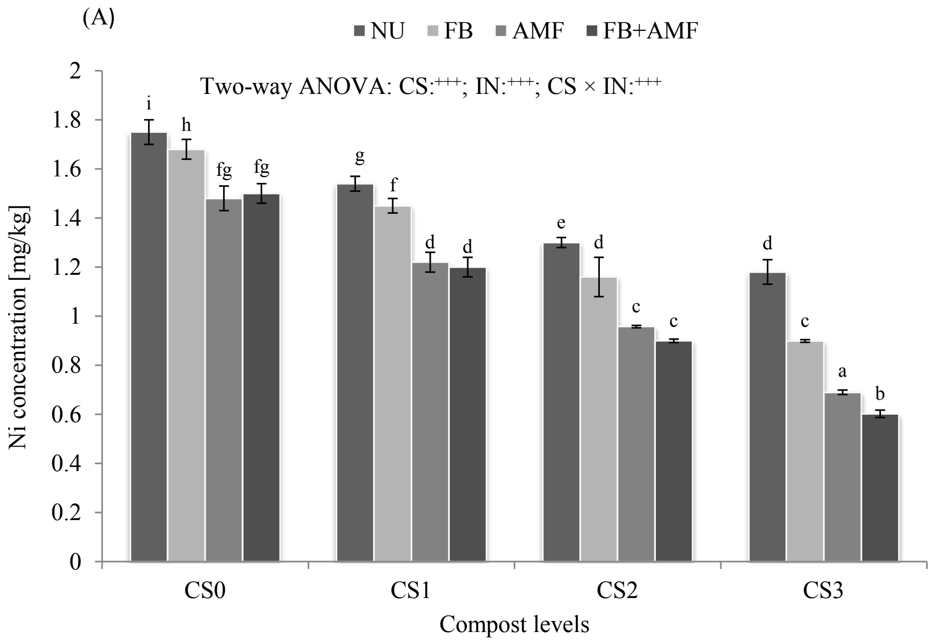
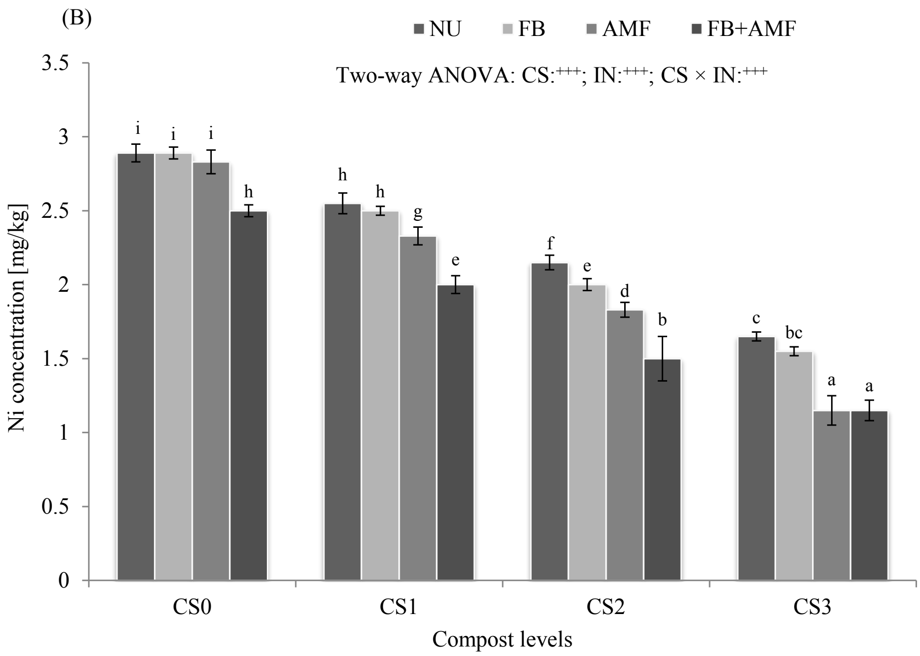
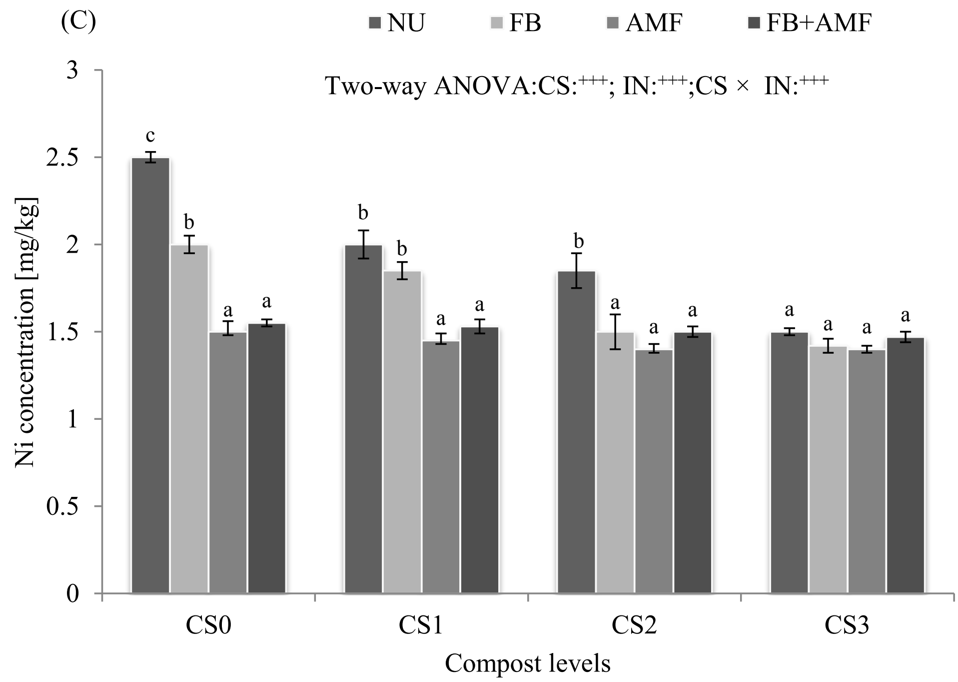


Figure 2. Effect and residual effect of compost and native microbes on mycorrhizal dependency [%] of (A) peas and (B) radish growth


(A)
NU FB AMF FB+AMF
Two-way ANOVA: CS:+++; IN:+++; CS × IN:+++
Ni concentration [mg/kg]
Compost levels
CS0
CS1
CS2
CS3


(B)
NU FB AMF FB+AMF
Two-way ANOVA: CS:+++; IN:+++; CS × IN:+++
Ni concentration [mg/kg]
Compost levels
CS0
CS1
CS2
CS3



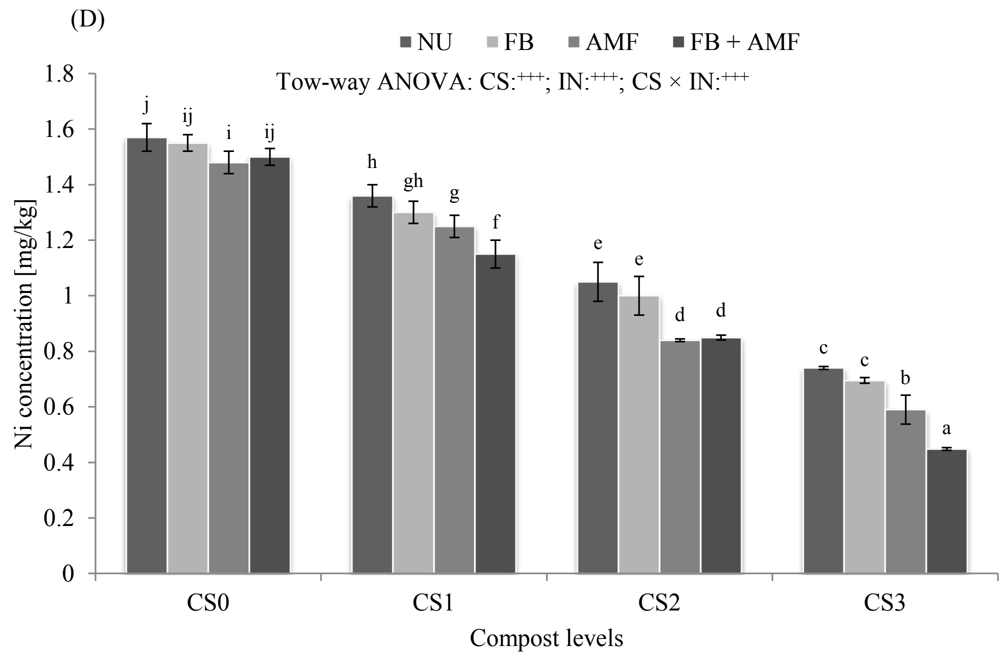
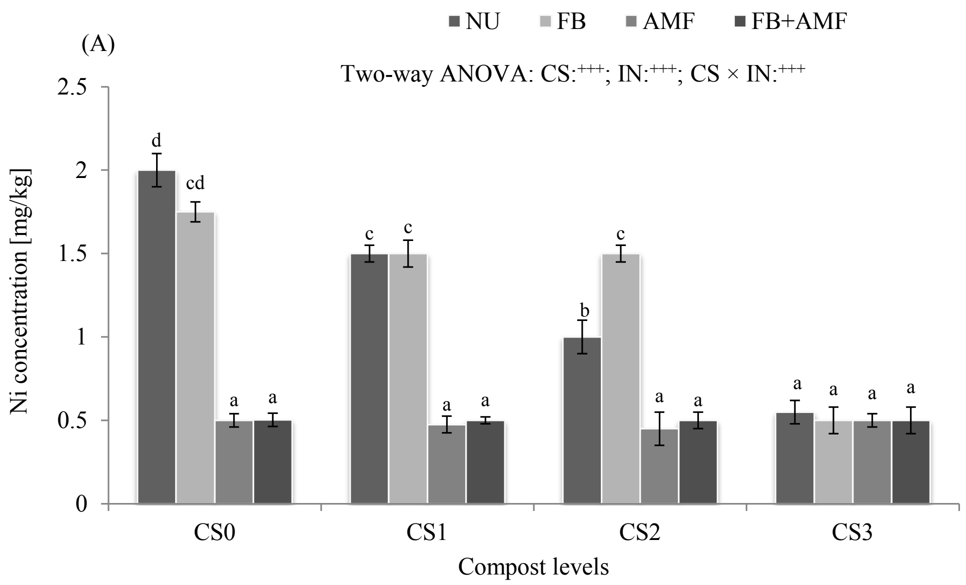


Figure 3. Ni concentration in pea plants [mg/kg, DW]. (A) roots, (B) shoots, (C) peels, (D) seeds. Mean ± SD (n=3). One-way ANOVA was performed for amendment treatments; Means for each parameter with different letters are significantly different from each other ($p < 0.05$) according to the Duncan test; Two-way ANOVA was performed to determine the effect of compost, inoculation and their interaction on polluted soil. NS not significant; $^{+}p < 0.05$; $^{++}p < 0.01$; $^{+++}p < 0.001$



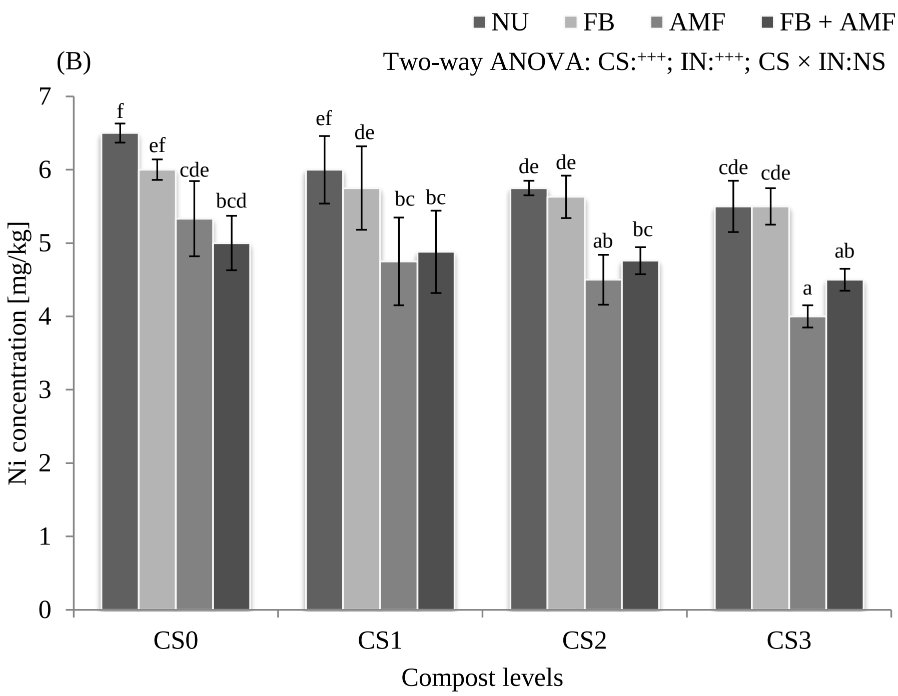
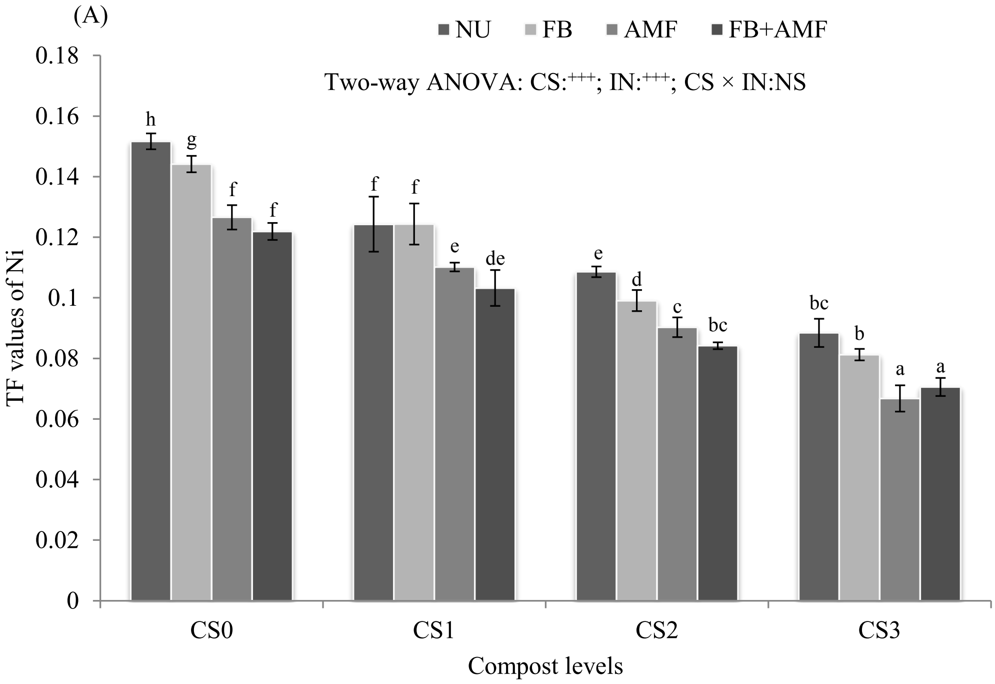


Figure 4. Ni concentration in radish plants [mg/kg, DW]. (A) roots and (B) shoots. Mean ± SD (n=3). One-way ANOVA was performed for amendment treatments; Means for each parameter with different letters are significantly different from each other ($p < 0.05$) according to the Duncan test; Two-way ANOVA was performed to determine the effect of compost, inoculation and their interaction on polluted soil. NS not significant; $^{+}p < 0.05$; $^{++}p < 0.01$; $^{+++}p < 0.001$



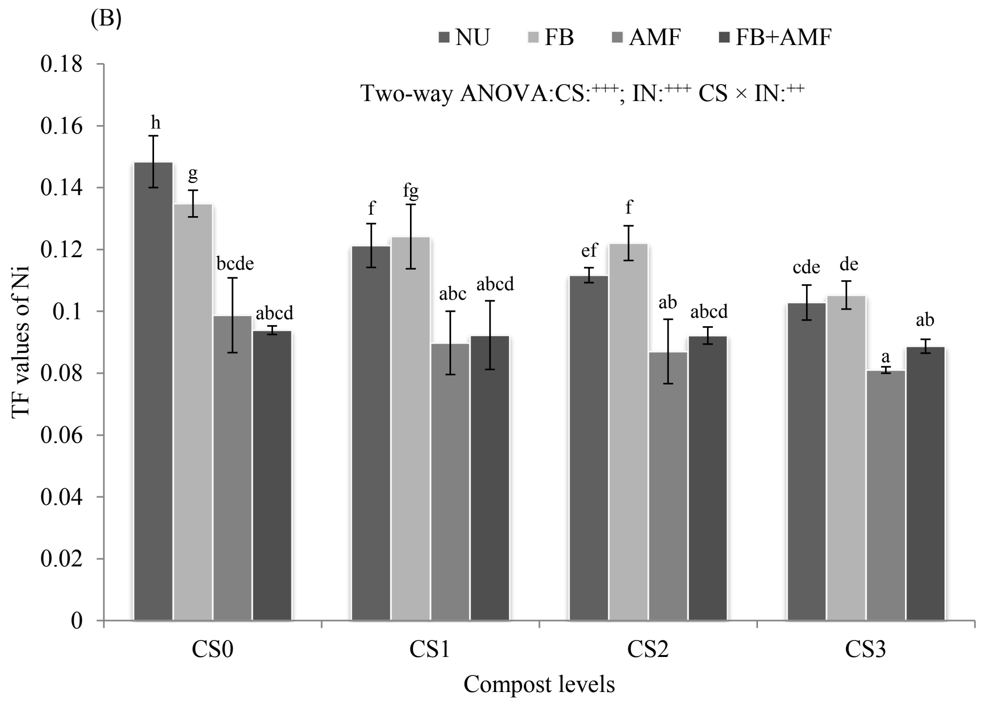


Figure 5. Transfer factor (TF) values of Ni in total plants of (A) pea and (B) radish. Mean ± SD (n=3). One-way ANOVA was performed for amendment treatments; Means for each parameter with different letters are significantly different from each other ($p < 0.05$) according to the Duncan test; Two-way ANOVA was performed to determine the effect of compost, inoculation and their interaction on polluted soil. NS not significant; $^{+}p < 0.05$; $^{++}p < 0.01$; $^{+++}p < 0.001$

maximum permissible level (0.01 mg/kg) recommended by WHO/FAO (2007). In general, results indicated that combination between compost and native microbes positively decreased Ni concentration in all plant organs compared to compost only and the control one. Several previous studies found that compost is a promising strategy to immobilize heavy metals in soils through changing the physicochemical property of soils and reacting with heavy metals (Liu *et al.* 2009; Bolan *et al.* 2014). Compost mainly immobilizes heavy metals through its humus substance, microorganisms, and inorganic components. Abundant humus substances in compost contain a large number of organic functional groups, such as carboxyl, carbonyl, and phenols, which can bind metal ions through complexation (Caporale *et al.* 2013; Tsang *et al.* 2014). The effectiveness of the fungal – bacterial inoculation on the reduction of Ni concentration may be explained on the basis of that microbes mobilize the heavy metals from the contaminated sites by leaching, chelation, methylation and redox transformation of toxic metals. Heavy metals can never be destroyed completely, but the process transforms their oxidation state or organic complex, so that they become water-soluble, less toxic and precipitated (Garbisu & Alkorta 2001). The positive decline of Ni in mycorrhizal plants of peas and radish may be due to the interaction effects between compost and AM fungi. Many research studies reported about the effect of AMF in improving plant tolerance to Ni stress which explained by decreasing metal accumulation and translocation to shoots (e.g., Vivas *et al.* 2006; Amir *et al.* 2013). As well as, Hildebrandt *et al.* (2007) suggested that AMF could filter out toxic metals by accumulating them in their mycelia. Two-way ANOVA revealed that the factor "compost" and "inoculation" had an extremely significant ($p < 0.001$) effect on Ni concentration for all organs of peas and radish. Although, the interaction of "compost × inoculation" factor had an extremely significant ($p < 0.001$) effect on Ni concentration for all organs of peas and radish but affected non-significantly on Ni content in the shoot of radish.

Transfer factor (TF) from soil to plants

Soil to plant transfer of heavy metals is the major pathway of human exposure to metal contamination (Jolly *et al.* 2013). Results in Figure 5A, B illustrate the transfer factor (TF) of Ni from polluted soil to pea and radish plants. Results observed that the TF values of different treatments varied from 0.067 to 0.152 for Ni by pea plants while varied from 0.089 to 0.148 for Ni by radish plants. The data showed that TF decreased significantly ($p < 0.001$) by increasing compost rates and combination between compost and inoculation. The minimum TF of Ni (0.067 and 0.089) was attained as a result of applying CS3 inoculated with AMF and its residual effect as a soil remediate polluted soil to peas and radish respectively. The seed vegetable (peas) is found to show a lower transfer factor for Ni than in root vegetable (radish). It was observed that TF for Ni in peas and radish less than 1. This means that these plants are not good bioaccumulators for Ni. The effect of applied compost levels and inoculation on decreasing TF values of Ni for peas and radish plants could be arranged in descending order of: CS+AMF ≥ CS+BF+AMF > CS+BF > CS. Two-way ANOVA indicated that the factor "compost" and "inoculation" extremely significantly ($p < 0.001$) affected on TF of Ni for the two tested plants but the factor interaction of "compost × inoculation" significantly ($p < 0.01$) affected Ni TF for radish plants and non-significant affected of Ni for pea plants.

CONCLUSIONS

The present study suggests that the combined application of compost and native microbes (*Bacillius* sp., *Aspergillus niger, A. terreus*, *Penicillium funiculosum, Fusarium culmorum Acaulospora bireticulata*, *Gigaspora margarita*, *Glomus lamellosum*, *Funneliformis mosseae*) was more effective in decreasing the availability of Ni in polluted soil. Also, our results indicated that compost with inoculation and their residual effects positively decreased Ni concentration in all plant organs compared to compost only and the control one. This could be important bio-resource for efficient bioinoculant development to enhance the tolerance of vegetables plants to heavy metal stress. Our findings indicate that compost and inoculation affected significantly the growth measurements of peas and radish. The current study recommends the use of compost and native microbes to provide a competitive advantage for remediation and pay attention concerning the industrial dusts and air fumes which contain toxic heavy metal to avoid their hazards impacts on environment.

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Received: April 4, 2017

Agriculture (Poľnohospodárstvo), 63, 2017 (2): 67−73
DOI: 10.1515/agri-2017-0006
Original paper

EFFECT OF ARBUSCULAR MYCORRHIZAL FUNGI ON CHEMICAL CONSTITUENTS IN COTTON/ALFALFA MIXED CULTURE

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IBRAHIM, M.: Effect of arbuscular mycorrhizal fungi on chemical constituents in cotton/alfalfa mixed culture. Agriculture (Poľnohospodárstvo), vol. *63*, 2017, no. 2, pp. 67–73.

A pot experiment was conducted to study the extent of changes occurring in the nutrients, chlorophyll and protein of plants grown in cotton/alfalfa mixed culture as affected by inoculation with indigenous arbuscular mycorrhizal fungi (AMF). The experiment consisted of mycorrhizal treatments (with and without AMF inoculation) and three planting patterns (cotton monoculture, alfalfa monoculture, cotton/alfalfa mixed culture). Arbuscular mycorrhizal (AM) inoculum previously isolated from a rhizospheric soil of cotton, was a mixture of *Glomus intraradices*, *G. viscosum*, and *G. mosseae*. Results showed that total chlorophyll and protein concentrations, and nutrients content were higher in AM cotton plants compared with the non-AM control. Mixed culture had a positive effect on all the above parameters in cotton shoot. The highest values were noted in AM plants in the mixed culture. Improved chemicals and biochemical constituents in cotton led to an increase in dry matter production. The highest dry matter was observed in the AM mixed culture, and was significantly higher by 1.4 times than that of non-AM monoculture.

Key words: cotton, AMF, alfalfa, mixed culture, chlorophyll, nutrients

Sustainable agriculture refers to correct agricultural resource management which it retains environmental quality and capacity of the soil and water resources (Reijntjes *et al.* 1992). Legume and non-legume intercropping cultivation has been widely encouraged in sustainable agriculture because it has the potential to allow plants to use soil nitrogen (N) more efficiently and improve the yield (Hauggaard-Nielsen *et al.* 2009; Gao *et al.* 2014). The productivity of agricultural lands can be improved in mixed culture system if the plants that are combined in this system do not compete each other for sunlight, water and nutrients. The plants which have the most differences in the use of resources, are compatible plants in intercropping (Vandermeer 1989).

Alfalfa is an important forage legume due to its nutritional quality, high protein content and adaptability to soil and climatic conditions (McDonald *et al.* 1991). Cotton is a mycotrophic plant (Siqueira *et al.* 1986) in which growth and nutrient uptake is usually increased by arbuscular mycorrhizal fungi (AMF) colonization. In sustainable agriculture, the AMF plays a key role in helping plants to efficiently recycle nutrients and thus remain productive under adverse conditions (Mosse 1986). AMF support N_2 fixation by legumes through providing plants with phosphorus and other immobile nutrients such as copper and zinc that are essential for N_2 fixation (Clark & Zeto 2000). The productivity of AM plants may be improved if micronutrient uptake is elevated, resulting in more competitive plants. Micronutrients are involved in biochemical and physiological functions such as biosynthesis of proteins, chlorophyll, and carbohydrate metabolism (Rengel 1999; Parmar *et al.* 2012). Also, the photosynthesis is affected by micronutrients through various modes of action. The increase of leaf chlorophyll content improves photo

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synthesis, and finally, the yield increases (Tilak *et al.* 1992). Elevated protein content by mixed culture (Herbert *et al.* 1984) could indicate the importance of this culture pattern in improving quality of crops.

AMF inoculation of legume/non legume mixed culture and the resulting changes in plant biochemicals and nutrients status, could influence the growth of mixed plants.

The objective of this research was to study the effect of inoculation with indigenous AMF on some chemical and biochemical constituents in cotton (main crop) and alfalfa (associated crop) plants grown in mono and mixed culture.

MATERIAL AND METHODS

The experiment was conducted during the summer season 2012 at Der-Alhajar research Station, southeast of Damascus, Syria (33°21' N, 36°28' E). The area is located within an arid region in which the total annual precipitation (in winter) is 120 mm. The average minimum temperature in winter is 1.3°C in January, increasing to an average of 36°C during August. Plastic pots were filled with 7 kg of dry topsoil (0–25 cm) collected from the field which had been planted with alfalfa. The soil was sandy clay with pH of 7.6, and contained 10.1 g/kg of organic matter, 0.9 g/kg of total N, 13.3 mg/kg of available N, 11.8 mg/kg of available P.

AM inoculum preparation

The arbuscular mycorrhizal inoculum including *Glomus intraradices* (Schenck & Smith), *G. viscosum* (Nicolson), and *G. mosseae* (Nicol. & Gerd.) Gerd. & Trappe, was previously isolated from a rhizospheric soil of cotton (Ibrahim 2010). For propagating AMF, pot-cultures were established by mixing original soil samples with sterilized sand as a substrate for growth of onion (*Allium cepa* L.) as the trap plant. Pot-cultures were conducted during two successive cycles of 4 months. The substrate containing onion root fragments, mycelium and spores were air dried and used as inoculum. The number of infective propagules in the inoculum was done by employing the most-probable-number technique (Sieverding 1991). The inoculum contained 560 propagules per 100 g of substrate.

Planting procedures

Seeds of cotton (*Gossypium hirsutum* L. Aleppo 33/1) and alfalfa (*Medicago sativa* L.) were surface sterilized by immersion in 20% NaOCl for 1 min before sowing. Five seeds of alfalfa and three seed of cotton were sown per pot. Half of the pots received the AM inoculum (100 g per pot) by layering at 10 cm depth of the pots at the time of sowing. Because of very low number of AM propagules in soil, soil for non-AM plants was not pasteurized. Each non-inoculated pot received the same amount of sterilized AM inoculum. Because abundant nodules have been previously observed on the roots of alfalfa grown at the field from which the soil was collected, the seeds were not inoculated with rhizobium. After emergence, the alfalfa seedlings were thinned to three plants per pot and the cotton seedling were thinned to one plant per pot. Plants were watered as needed until sampling date.

Experiment design and treatments

The experiment was designed into a randomized complete block design with four replicates. The experiment consisted of two treatments of mycorrhiza (with and without) and three planting patterns (cotton monoculture, alfalfa monoculture, cotton-alfalfa mixed culture). The experiment involved six treatments: (i) cotton monoculture, (ii) cotton monoculture with AMF, (iii) alfalfa monoculture, (iv) alfalfa monoculture with AMF, (v) cotton/alfalfa mixed culture, and (vi) cotton/alfalfa mixed culture with AMF.

Determination of chlorophyll concentration

To determine the concentrations of chlorophyll a (Chl a), chlorophyll b (Chl b), and total chlorophyll in cotton leaves, discs (100 mg) were cut from the upper-most fully expanded leaves randomly selected from three plants of each treatment. Discs were macerated in 5 mL acetone (80%) for 24 h. The absorbance of the pooled extracts was measured at 663 and 645 nm using a spectrophotometer (Thermo Spectronic, UK). The concentrations of Chl a, Chl b, and total chlorophyll were determined using the equations of Arnon (1949).

Plant sampling and analysis

All plants were sampled by cutting stems at soil level. Plants of both species were sampled at the flowering stage of cotton. Above-ground biomass was measured after drying to a constant weight at 70°C. Crude protein was estimated by multiplying 6.25 by total N which was measured using micro-Kejeldahl method. Ca, K, Fe, Zn, Cu, and Mn were determined by X-ray fluorescence (XRF) (Khuder *et al.* 2009). The determination of phosphorus was based on the colorimetric method first proposed by Misson (1908) using spectrophotometer (ThermoSpectronic, UK).

Determination of mycorrhizal colonization

Roots samples were rinsed, cut into 1 cm fragments, totally mixed, cleared with KOH and stained with acid fuchsine in lactoglycerol. The percentage of root colonization was determined microscopically using a gridline intercept method (Giovannetti & Mosse 1980).

Statistical Analysis

The data were analysed by the analysis of variance (ANOVA) using SAS program (SAS Institute Inc, 2004), and means were compared by the least significant difference (Fisher's PLSD) test at a confidence level of 5%.

RESULTS

Mycorrhizal colonization

Root colonization by AMF occurred in all treatments including non-inoculated plants (Table 1). The latter plants showed a low colonization level (less than 2%). Percent AMF colonization of cotton was significantly increased when plants were grown in mixed culture (Table 1). The root colonization was 33.8 and 35.5% in mono and mixed culture, respectively.

Chlorophyll and crude protein concentrations

The effects of AMF and pattern of culture were significant on cotton chlorophyll a and total chlorophyll concentration (Table 1). Higher concentrations of chlorophyll a and total chlorophyll were observed in AM plants compared to non-AM plants. Also, the concentrations of chlorophyll a and total chlorophyll were higher in mixed culture than that of monoculture regardless of AMF inoculation. The maximum chlorophyll value was obtained in AM mixed culture with mean of 9.93, and 14.3 mg/g fresh matter for chlorophyll a and total chlorophyll, respec-

T a b l e 1

Effect of AMF and planting pattern on AMF colonization, above ground dry matter (DM), crude protein and chlorophyll (a, b, total) concentrations, and chlorophyll ratio a/b

Planting pattern	AMF inoculation	AMF [%]	DM [g/pot]	Protein [mg/g DM]	Chl a	Chl b	Total chl	a/b
					[mg/g FM]			
Cotton monoculture	Non-AMF	1.5^{c}	6.52^{d}	10.20^{d}	8.05^{c}	3.68^{b}	11.73^{c}	2.19^{c}
Cotton monoculture	AMF	33.8^{b}	11.81^{b}	10.85^{c}	9.54^{b}	3.65^{b}	13.19^{b}	2.61^{a}
Cotton mixed culture	Non-AMF	1.7^{c}	9.94^{c}	11.65^{b}	9.61^{b}	3.89^{b}	13.50^{b}	2.49^{ab}
Cotton mixed culture	AMF	35.5^{a}	15.67^{a}	13.51^{a}	9.93^{a}	4.38^{a}	14.30^{a}	2.27^{bc}
$LSD_{(0.05)}$		1.4	0.62	0.31	0.21	0.42	0.45	0.27
Alfalfa monoculture	Non-AMF	1.6^{b}	1.21^{b}	15.69^{d}	–	–	–	–
Alfalfa monoculture	AMF	27.0^{a}	2.27^{a}	17.54^{c}	–	–	–	–
Alfalfa mixed culture	Non-AMF	1.4^{b}	1.07^{b}	18.65^{b}	–	–	–	–
Alfalfa mixed culture	AMF	25.7^{a}	2.17^{a}	20.33^{a}	–	–	–	–
$LSD_{(0.05)}$		1.7	0.14	0.87	–	–	–	–

Means within a column followed by different letters are significantly different ($P < 0.05$); *LSD* – least significant difference; FM – fresh matter tively (Table 1). Chlorophyll ratio a:b decreased in AM plants grown in mixed culture (Table 1). AM mixed culture showed higher concentrations of crude protein in comparison with the other treatments (Table 1). Higher protein concentration was observed in alfalfa compared to that in cotton in all treatments. The maximum protein values were observed in AM mixed culture with mean of 20.33 and 13.51 mg/g in alfalfa and cotton, respectively (Table 1).

Cotton and alfalfa shoot dry matter

AMF inoculation increased significantly shoot dry matter of cotton regard less of culture pattern (Table 1). Cotton shoot dry matter in the mixed culture was significantly higher than that of monoculture whether inoculated or not. The highest shoot dry matter of cotton was observed in the AM mixed culture, and was significantly higher by 1.4 times than that of non-AM monoculture treatment (Table 1). AMF also significantly improved the dry matter of the alfalfa shoots compared to the non-AM treatments (Table 1). Alfalfa dry matter was significantly higher by 0.8 times in AM mixed culture than that of non-AM monoculture.

P, K, Ca and selected micronutrients content

AMF produced significant increase in P content of cotton and alfalfa regardless of culture pattern (Table 2). Also, AMF inoculation increased the content of K, Ca, Mn, Fe, Cu, and Zn in shoot of cotton and alfalfa in both monoculture and mixed culture (Table 2). Mixed culture increased all selected nutrients content in cotton shoot. The highest values were observed in the AM mixed culture (Table 2). In contrast, K, Ca and selected micronutrients content in alfalfa shoot decreased in AM mixed culture compared to AM monoculture (Table 2).

DISCUSSION

The colonization rate achieved by AMF, might indicate adaptation of the indigenous fungi to their native soils in the sense of benefiting their hosts best under these given soil conditions. Numerous researches showed that AMF differ in their ability to inoculate particular plant species and that AMF tend to exhibit the highest response in their soils of origin (Bohrer *et al.* 2003; Klironomos *et al.* 2003). The present study showed that AMF colonization was increased when cotton plants were grown in mixed culture. This result agrees with that of Derelle *et al.* (2015) who showed a positive influence of mixed cultures on AMF root colonization compared with monoculture.

T a b l e 2

Effect of AMF and planting pattern on nutrients content in cotton and alfalfa shoot

Treatment	P	K	Ca	Mn	Fe	Cu	Zn
	Nutrient content in cotton [mg/nutrient/pot]						
Monoculture	17.01[d]	147.3[d]	183.3[d]	0.3584[d]	1.4435[d]	0.029[c]	0.1984[d]
Monoculture +AMF	39.52[b]	282.5[b]	367.5[b]	0.8272[b]	3.2587[b]	0.064[b]	0.3638[b]
Mixed culture	26.87[c]	206.5[c]	239.1[c]	0.5364[c]	2.2192[c]	0.043[b]	0.2604[c]
Mixed culture +AMF	59.73[a]	320.6[a]	418.0[a]	1.2121[a]	4.4350[a]	0.077[a]	0.4194[a]
$LSD_{(0.05)}$	2.85	18.64	19.78	0.24	0.58	0.01	0.05
Nutrient content in alfalfa [mg/nutrient/pot]							
Monoculture	2.52[b]	18.5[c]	16.1[c]	0.0571[c]	0.5968[c]	0.0077[c]	0.0279[c]
Monoculture +AMF	5.55[a]	51.3[a]	37.5[a]	0.1619[a]	1.6833[a]	0.0145[a]	0.0684[a]
Mixed culture	2.28[b]	14.2[d]	12.6[d]	0.0539[c]	0.4910[c]	0.0050[d]	0.0253[c]
Mixed culture +AMF	5.62[a]	29.3[b]	31.5[b]	0.1282[b]	1.1497[b]	0.0126[b]	0.0602[b]
$LSD_{(0.05)}$	0.26	2.4	2.2	0.01	0.15	0.001	0.007

Means within a column followed by different letters are significantly different ($P < 0.05$); *LSD* – least significant difference; Nutrient content [mg/pot] = nutrient concentration [mg/g] × dry matter.

Higher P content in AM plants observed in this study, agrees with earlier studies (Smith & Read 1997; Clark & Zeto 2000). AMF might increase P acquisition of plant by enhanced exploration of soil P not available to plant roots by the external mycelium, and increase P solubilisation by AM root exudates which resulted in an increase in P content of soils. The ability of AMF to increase plants uptake of Cu, Mn, Zn and Fe is well documented (Liu *et al.* 1994; Ibrahim 2010). The increase in micronutrients absorption might be due to a better exploration of the soil by the AM hyphae and to a greater P and water uptake of AM plants (Davies *et al.* 2005; Smith & Read 2008). On the other hand, AMF tend to produce more CO_2 in the rhizosphere soil (Mohammad *et al.* 2005), causing an decrease in soil pH and eventually the availability of nutrients. Higher nutrients content achieved by mixed culture compared to monoculture, may indicate that the mixed culture used plant growth resources more efficiently than sole crop (Fukai & Trenbath 1993). The decrease in selected nutrients content in alfalfa compared to cotton was partly a reflection of differences in dry matter. Plants facilitation and/or competition may depend on the identity and diversity of AMF species (Walder *et al.* 2012), the size and age of the plants (van der Heijden & Horton 2009), and the supply levels of important nutrients. Higher nutrients content in cotton may indicate that cotton can be more competitive than alfalfa in soils where nutrients availability is reduced. The higher competitive ability might be due to the greater root length of cotton and faster root growth compared to alfalfa. The complementarity resource use between cotton and alfalfa could be enforced by AMF resulting in competitive enhancement of AM cotton. The lower nutrients contents in mixed alfalfa could be due to the decrease in the nutrients concentrations in the rhizosphere of alfalfa plants, leading to markedly decreased content in tissues.

The chlorophyll content in a plant depends on the ability of nitrogen adsorption by the plant, and this is considered an important factor in farm management (Jongschaap & Booij 2004). In the present study, the increase in chlorophyll concentration of cotton leaf in mixed culture compared to monoculture could be attributed to the increase in nitrogen fixation by alfalfa and to higher usage of available nitrogen in the soil. Many studies showed higher leaf chlorophyll content in the intercropping than monoculture (Tsubo *et al.* 2005; Ghosh *et al.* 2006). According to Ghosh *et al.* (2006), the higher chlorophyll content of sorghum leaf in intercropping of soybean/sorghum was attributed to the overcast of plants on each other and nitrogen fixation by legume in intercropping. On the other hand, improved micronutrients uptake play an important role in biosynthesis of chlorophyll, proteins and secondary metabolites (Rengel 1999; Subramanian *et al.* 2009; Parmar *et al.* 2012). Also, providing legume with P and other immobile nutrients could support nitrogen fixation of legume (Clark & Zeto 2000). Therefore, the transfer of fixed N to cotton may contribute improvement of chlorophyll content in cotton. Decreased chlorophyll ratio a:b in AM mixed culture was due to increase of chlorophyll b, which might be due to increased plants shading on one another. Under the shade conditions, plants tend to increase their content of chlorophyll to trap more light.

It seems that AMF has a role in increasing the availability of cotton to nitrogen sources and increasing the protein concentration. Increased protein concentration in mixed culture is in agreement with the results of Herbert *et al.* (1984) and Najafi *et al.* (2013) who showed that protein content were higher in mixed compared to monoculture. Fixing of atmospheric nitrogen by alfalfa and its transfer to cotton can increase protein in the mixed culture. Many researches showed that the fixed nitrogen is transferred to intercrop (Xiao *et al.* 2004; Wahbi *et al.* 2016). Both nitrogen transfer (fixed nitrogen and residual nitrogen) can reduce the cost of the nitrogen supply in legume-based intercropping different systems (Willy 1990). Increased crude protein by AM mixed culture indicates the importance of this culture pattern in improving quality of alfalfa as animal feed. The dry matter weight of cotton grown in mixed culture was greater than monoculture. Experimental results obtained by Kulandaivel *et al.* (2001) reported that dry matter production of cotton was significantly higher in cotton/blackgram intercropping than sole cropping. Highest cotton dry matter values found in AM mixed culture is in agreement with the results of Pellegrino and Bedini (2014). This may be attributed to the improvement of nutrient uptake (Jia *et al.* 2004). Improving uptake of micronutrients by AMF could induce plant chlorophyll content and photosynthesis, and consequently, increase biomass production (Tilak *et al.* 1992).

CONCLUSIONS

The results clearly showed that the application of AMF in cotton/alfalfa mixed culture had a positive effect on the chemicals and biochemical constituents in cotton shoot. Enhanced chlorophyll and protein concentration of AM cotton plants can be attributed to enhanced mineral nutrition by AMF and the effect of alfalfa on nitrogen status in cotton. Improved chemicals and biochemical constituents in cotton led to an increase in the dry matter production.

Acknowledgements. The author is grateful to the Atomic Energy Commission of Syria (AECS) for encouragement and technical support.

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Received: April 3, 2017

DE GRUYTER OPEN

Agriculture (Poľnohospodárstvo), 63, 2017 (2): 74−85

Original paper DOI: 10.1515/agri-2017-0007

CURRENT STATE AND DEVELOPMENT OF LAND DEGRADATION PROCESSES BASED ON SOIL MONITORING IN SLOVAKIA

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KOBZA, J. – BARANČÍKOVÁ, G. – MAKOVNÍKOVÁ, J. – PÁLKA, B. – STYK, J. – ŠIRÁŇ, M.: Current state and development of land degradation processes based on soil monitoring in Slovakia. Agriculture (Poľnohospodárstvo), vol. *63*, 2017, no. 2, p. 74–85.

Current state and development of land degradation processes based on soil monitoring system in Slovakia is evaluated in this contribution. Soil monitoring system in Slovakia is consistently running since 1993 year in 5-years repetitions. Soil monitoring network in Slovakia is constructed using ecological principle, taking into account all main soil types and subtypes, soil organic matter, climatic regions, emission regions, polluted and non-polluted regions as well as various land use. The result of soil monitoring network is 318 sites on agricultural land in Slovakia. Soil properties are evaluated according to the main threats to soil relating to European Commission recommendation for European soil monitoring performance as follows: soil erosion and compaction, soil acidification, decline in soil organic matter and soil contamination. The most significant change has been determined in physical degradation of soils. The physical degradation was especially manifested in compacted and the eroded soils. It was determined that about 39% of agricultural land is potentially affected by soil erosion in Slovakia. In addition, slight decline in soil organic matter indicates the serious facts on evaluation and extension of soil degradation processes during the last period in Slovakia. Soil contamination is without significant change for the time being. It means the soils contaminated before soil monitoring process this unfavourable state lasts also at present.

Key words: soil monitoring, threats to soil, soil degradation processes, soils in Slovakia

The main aim of this contribution is to obtain the knowledge of the most current state and development of soil properties according to concrete threats to soil (soil erosion, soil compaction, soil acidification, decline in soil organic matter, soil contamination) on the basis of soil representative monitoring system in Slovakia, which consists of 318 monitoring sites where all soil types, geology, climatic regions, various land use – arable land, grassland and protected areas are included and what is in harmony with European strategy of soil monitoring. There are permanently monitored important parameters in connection to recommendation of EC for evaluation of current state and development of soils (van Camp *et al*. 2004) with regard to quantitative and qualitative parameters of soils (Kobza *et al.* 2014) concerning land degradation processes. Land degradation is a decrease in the optimum functioning of soils in ecosystems (de Kimpe & Warkentin 1998). The objective of this contribution is to offer objective information on current state and development of important soil parameters according to main threats to soil as significant component of environment (Linkeš *et al.* 1997). The obtained results from agricultural

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land are evaluated in this paper. In addition, forest land is a part of other monitoring system – Monitoring of forest ecosystems, which is developed by National Forestry Centre – Research Institute in Forestry in Zvolen.

Finally, obtained important current soil outputs are regularly imported to JRC (Joint Research Centre) in Ispra (Italy) and to EEA (European Environment Agency) in Copenhagen (Denmark), as well.

MATERIAL AND METHODS

Monitoring network

Soil monitoring system in Slovakia has been running consistently since 1993 year. Soil monitoring network in Slovakia is constructed on ecological principles and includes the research data of all main soil types and subtypes, soil substrates, climatic regions, emission regions, polluted and non-polluted regions as well as various land use (Figure 1). There are 318 monitoring sites on agricultural land in Slovakia. All soil monitoring sites are located in WGS 84 coordinates. These ones are permanently monitored and evaluated since 1993 in 5-years repetitions. We have completely finished 4 monitored cycles (1st – 1992–1996, 2nd – 1997–2001, 3rd – 2002–2006 and 4th – 2007–2012) for the time being. Nowadays, the 5th monitored cycle is still running and will be completely finished and evaluated in 2018 year soonest. Therefore the latest complete results on current state and land degradation processes development are evaluated in this contribution.

The monitoring site represents the circular shape, with a radius of 10 m and an area of 314 m^2. The standard depths of 0–0.10 m, 0.20–0.30 m and 0.35–0.45 m on soils under grassland and 0–0.10 m and 0.35–0.45 m on arable land are sampled, but the depth is adjusted to characterize the main soil horizons. The most important soil indicators concerning threats to soil are included in the soil monitoring system in Slovakia according to the recommendation of the European Commission (EC) for united soil monitoring system in Europe (van Camp *et al*. 2004).

Monitored indicators and analytical procedures

The most important soil indicators concerning threats to soil are included in soil monitoring system in Slovakia according to Recommendation of European Commission (EC) for unified soil monitoring system in Europe (van Camp *et al*. 2004). *Soil erosion (on selected soil transects)*: ^{137}Cs (gamaspec-

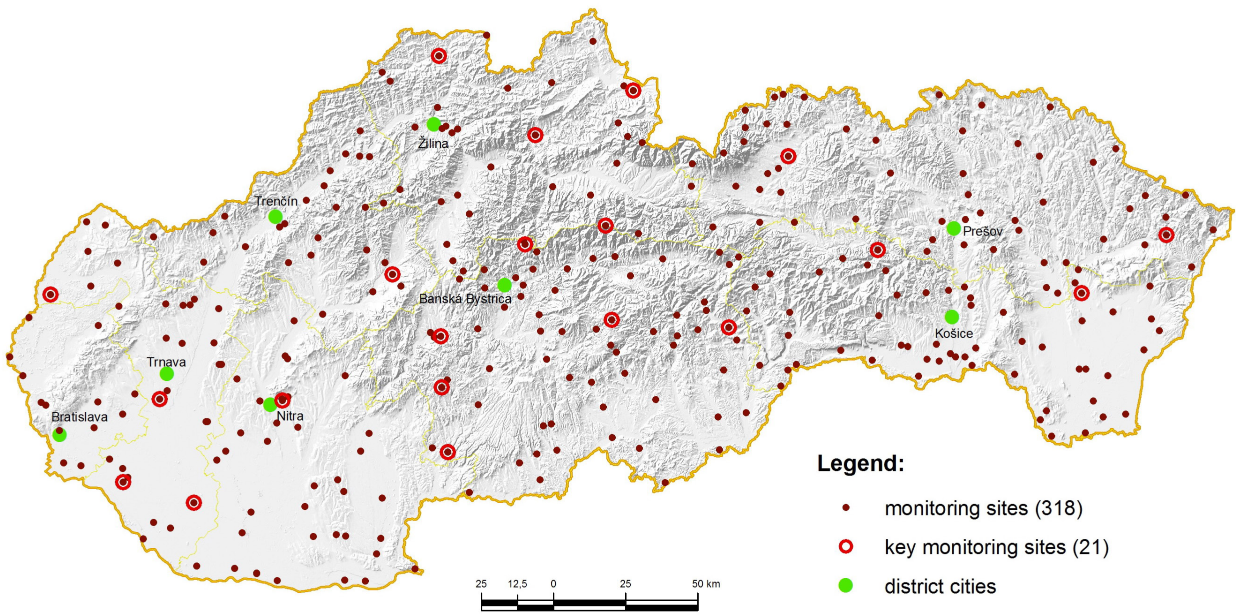


Figure 1. Soil monitoring network in Slovakia

trometrically with high resolution); pH/KCl; SOC (soil organic carbon; dry way using CN analyser); P (according to Egner´s method), K (according to Schachtschabel´s method); texture (according to FAO); s*oil compaction*: bulk density (ςd); porosity (P); maximum capillary water capacity (wKMK) in 100 cm^3 cylinders; texture (according to FAO); s*oil acidification*: pH/H_2O, active Al (according to Sokolov); *quantitative and qualitative composition of soil organic m*atter: SOC, Nt (total nitrogen; dry way using CN analyser), C_{HA}/C_{FA}, Q^4_6 (C_{HA}/C_{FA} carbon ratio of humic and fulvic acids, optical parameter Q^4_6 ratio of optical density of C_{HA} solution measured at 465 and 665 nm; according to Kononova and Beľčikova (ex. Kobza *et al.* 2011); s*oil contamination*: Cd, Cr, Pb, Ni, Zn, Cu, As Se, Co (extracted with aqua regia), Hg (total content, analyser AMA 254).

All described methodical and analytical procedures in more details were realized according to the work Uniform analytical procedures for soil (Kobza *et al*. 2011).

Evaluation of measured data

Statistical analysis and data evaluation was carried out using the program Statgraphic XV. Centurion.

RESULTS AND DISCUSSION

Soil erosion

Soil erosion belongs to the most environmental problems and the most extended degradation process in Slovakia. The process of soil erosion is significantly accelerated by unreasonable human activities in the current period of intensification of agriculture. Erosion is measured on erosive transects (series of pedological sites located down the slope) using ^{137}Cs profile distribution. This method was also used by other authors in conditions in Slovakia (Linkeš *et al*. 1992; Fulajtár & Janský 2001; Stankoviansky 2003; Styk 2005; 2007). In addition, the area of soil erosion distribution and its intensity is determined by using of the erosive predictive model where the USLE (Universal soil loss equation) is included (Wischmeier & Smith 1978). This interactive and predictive erosive model has been created for farmers on the purpose to obtain very helpful information on soil erosion intensity and its area distribution as well as to select and finally to implement appropriate methods of land management (Styk *et al.* 2009). They can find this model on http://www.podnemapy.sk.

The obtained results on potential water erosion on agricultural soils in Slovakia are given in Figure 2 and Table 1. On the basis of obtained results (Table 1) about 39% of agricultural soils registered in LPIS (Land Parcel Identification System) are potentially affected by water erosion in Slovakia. It means approximately 10% in medium, 15% in high and 14% in extremely high level (% of agricultural land). Regarding individual soil types, categories of extremely high to medium erosion predominates on soil types located at the mountainous and submountainous regions (Cambisols, Rendzic Leptosols) where about 75% of total area each of these soil types can be affected by potential water erosion.

Soil compaction

Soil compaction is monitored in the soil monitoring network only on arable land on the basis of measured physical indicators such as bulk density, porosity and texture. The values of bulk density on agricultural soils of Slovakia are mostly running between 1.2–1.6 g/cm^3 and are evaluated in the context of their critical values. Risk of soil compaction expressed as percentage of compacted monitoring sites from all evaluated soil types and soil texture is presented in the Figure 3 and 4.

Rate of soil compaction depends mainly on soil texture (significant differences between texturally medium heavy and heavy soils), the type of crop and its cultivation management (combination of crops concerning soil types and textures in sampling cycles) and weather conditions during preparation of soil and crops sowing as well as spring months (partially differences between sampling cycles). Other authors also report the influence of soil texture fractions (Heuscher *et al*. 2005; Benites *et al*. 2007; Shiri *et al*. 2017), the humus content (Soane 1990; Kummar *et al*. 2012), the different tillage and residual practice (Dam *et al*. 2005; Veiga *et al.* 2008) or soil moisture (Timm *et al*. 2006; Logsdon 2012) on soil compaction. Differences in topsoil compaction between soil types are not very significant. Suuster *et al*. (2011) state similar results in humus horizon of soils concerning Estonian soil monitoring. Higher rate of compaction was observed in intensively cultivated Chernozems, heavy Fluvisols and heavy Cambisols probably as a result of reduced tillage at some crops of crop rotation or in texturally differentiated soils (Albic Luvisols and Planosols). Rate of soil compaction is increasing in direction from texturally loamy to clayey soils which are the most sensitive to soil compaction. For the soil compaction development, the best soil physical conditions were recorded in the last monitoring cycle (except medium heavy Luvisols) particularly in relation to content of humus development (Figure 7). The worst state of soil physical properties at the most soil types were observed in the 2. cycle and improved to the last cycle. It likely relates also to the increase of soil organic carbon (Figure 3 and 4). According to soil types distribution the sensitive soils to soil compaction are situated mostly on southwestern and southeastern parts of Slovakia in the lowlands used mostly as agricultural – arable soils (loamy to clayey) cultivated by heavy machinery.

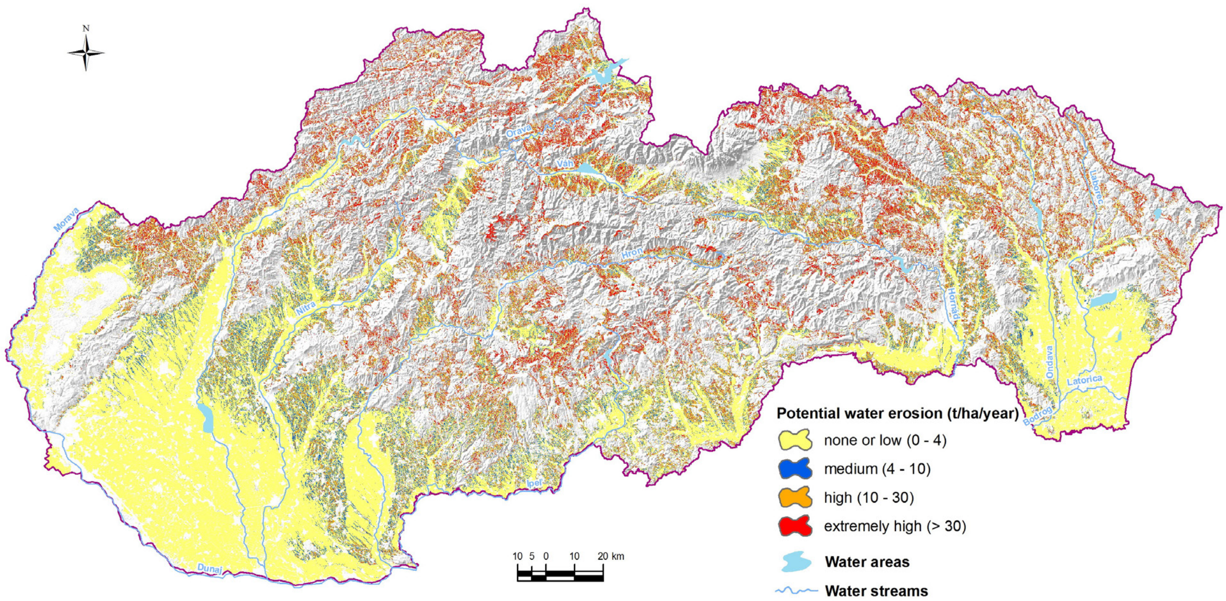


Figure 2. Potential water erosion on agricultural soils in Slovakia

T a b l e 1

Dominant agricultural soils [registered in LPIS] affected by potential water erosion in Slovakia

Soil type	Total area of soil type [ha]	Erosivity categories – soil loss [% of total area of soil type]			
		None or low (0–4 [t/ha/year]	Medium (4–10 [t/ha/year]	High (10–30) [t/ha/year]	Extremely high (more than 30 [t/ha/year])
Chernozem	286,861	95.4	3.2	1.2	0.2
Luvisol	251,209	58.9	19.5	17.5	4.1
Cambisol	864,749	24.1	9.3	23.6	43.0
Planosol, Luvisol	235,955	51.3	19.4	21.3	8.0
Rendzic Leptosol	103,631	24.4	7.9	22.3	45.4
Total area of agricultural soils	1,985,535	61.2	10.4	14.8	13.6

Soil acidification

Soil acidification, normally indicated by the pH decline of a certain soil, has recently received increasing attention due to its important impact on soil environmental quality, food security and human health (Godfray *et al.* 2010). The optimal value of the pH value is the key aspect in soil quality evaluation that belongs to the dynamic soil parameters. It means, that the slight changes of this parameter influence the potential of soil sorbents and inorganic contaminants mobility and their transport (Borůvka & Drábek 2004; Makovníková *et al*. 2007; Jones *et al*. 2012). In the 4th monitoring cycle (sampling in 2007) the decrease of average of active pH value (compared with 1993) in 9 groups of soils was recorded in the depth of 0–10 cm (Figure 5).

The most significant pH reduction in arable land was recorded in the group of Dystric Fluvisols, in grassland in Haplic Podzols group, Skeletic Leptosols and Lithic Leptosols group and the Planosols group. The acidification process in dystric Fluvisols, located on alluvial deposits (along Váh, Hron and Bodrog rivers), decreases their ability to the immobilisation of the potential risk element just due

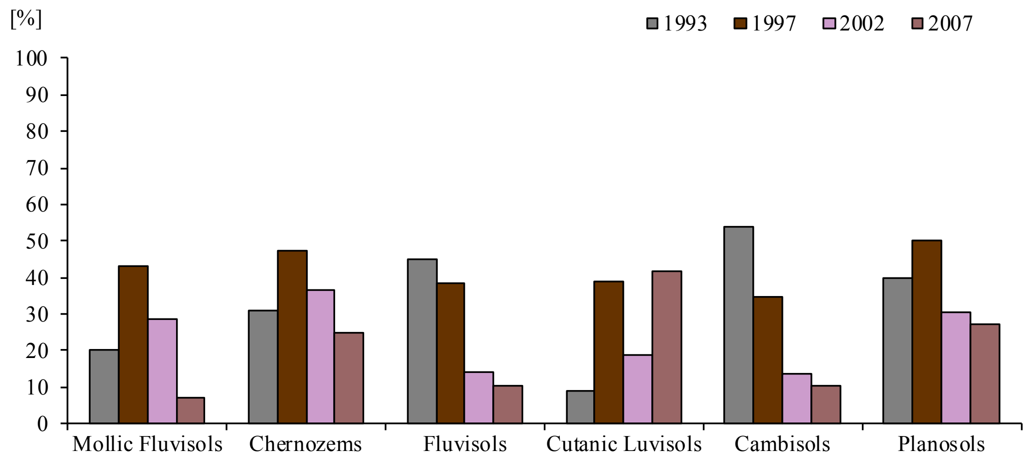


Figure 3. Risk of soil compaction [% of compacted monitoring sites] for main soil types – texturally medium heavy soils in Slovakia

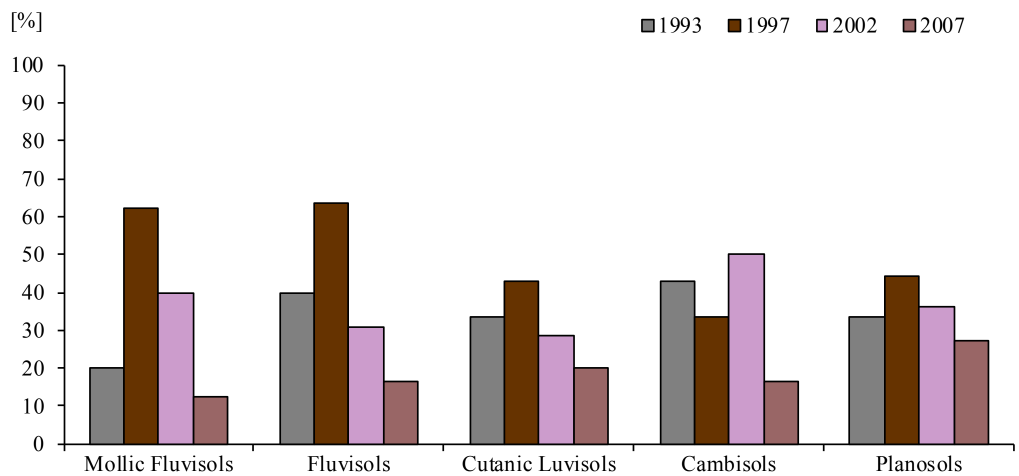


Figure 4. Risk of soil compaction [% of compacted monitoring sites] for main soil types – texturally heavy soils in Slovakia

low pH value of soils as well as low content of soil organic matter. Soils with very low immobilisation potential are reported predominantly in Košice region and Banská Bystrica region, where soils are contaminated by geochemical anomalies and anthropogenic sources, as well (Čurlík & Šefčík 1999; Makovníková *et al.* 2007; Fazekašová *et al.* 2016).

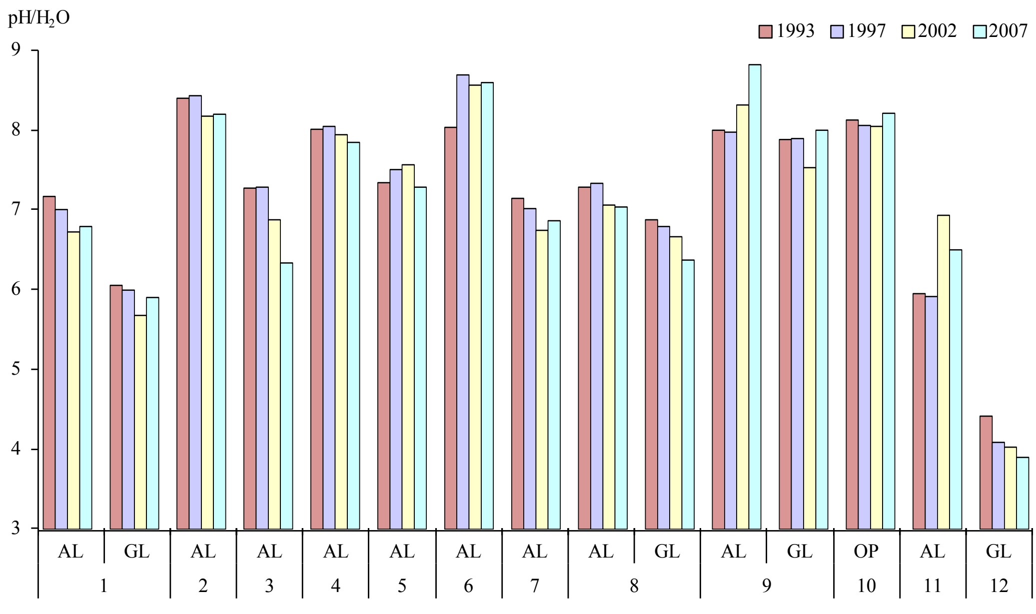
The active Al content in context with pH value is monitored. Al toxicity to plants manifests from slightly acidic to acidic soils. High Al concentrations as Al^{3+} represent an important growth and yield limiting factor for crops in acid soils (pH in $H_2O \leq 6.0$) (Makovníková & Kanianska 1996; Mačuha 1999; Meriño-Gergichevich 2010).

Determined negative correlation between pH value and active Al content ($r = -0.77$) (Figure 6) in soil is consistent with the work of various authors (Hiradate 2004; Meriño-Gergichevich 2010) and highlights the potential danger of acidification. The increase of the active aluminium content (between the first and the last monitoring cycles) was determined in the group of Podzols, Lithic Leptosols and Skeli-Dystric Leptosols used as permanent grassland as well as in the group of Cambisols on the crystalline rocks and in the group of Dystric Fluvisols used as arable land. These results are consistent with pH value trends during the observed period. Increased attention must be given high maximum values of active aluminium on arable land, which can significantly inhibit the growth of cultivated crops and subsequently to contaminate the nutrient chain.

Decline in soil organic matter

Soil organic matter represents more than 95% of total carbon accumulated on soils under grassland and near 100% of total carbon accumulated on arable land (Stolbovoy & Montanarella 2008). Quantitative and qualitative indicators of soil organic matter (SOM) are permanently monitored in soil monitoring network in Slovakia. Originally, after slight decline in soil organic carbon (SOC) on the beginning of soil monitoring system in Slovakia (1993 year), its increase has been indicated on all arable soils during last period (Figure 7).

There are many reasons of SOM decline as intensive conventional soil cultivation (Aranda *et al*. 2011), deep plowing (Caurasano *et al*. 2006), incorrect crop rotation (Machado *et al*. 2006), or insuficient supply of quality organic matter (Stetson *et al*. 2012). In Slovak agriculture reason of SOM decline



FAO – WRB 2015: 1– Cambisols, 2 – Calcaric Fluvisols, 3 – Fluvisols, 4 – Chernozems, 5 – Luvisols, 6 – Phaeozems, 7 – Calcaric Phaeozems, 8 – Albic Luvisols and Planosols, 9 – Rendzic Leptosols, 10 – Calcaric Regosols, 11 – Regosols, 12 – Podzols, AL – Arable land, GL – Grassland

Figure 5. Comparison of pH values in topsoil of arable soils of Slovakia

on his beginning of soil monitoring system could be sharply drop of organic fertiliser consumption (Bielek 2014). Also increase of SOM concentration or maintance on sufficient level of SOM can be caused by several methods of good agricultural practice, as application of quality organic fertilisers, adherence to optimal crop rotation and minimal soil cultivation (Janzen 2006; Abdalla *et al*. 2013). In our case increase of SOM in next monitoring period it could be probably caused by subsidies of Slovak Government for increasing of soil organic matter in soils.

Qualitative indicators (C_{HA}/C_{FA} and Q^4_6 ratio) of soil humus are without significant trends. However, the measured values are characteristic for the con-

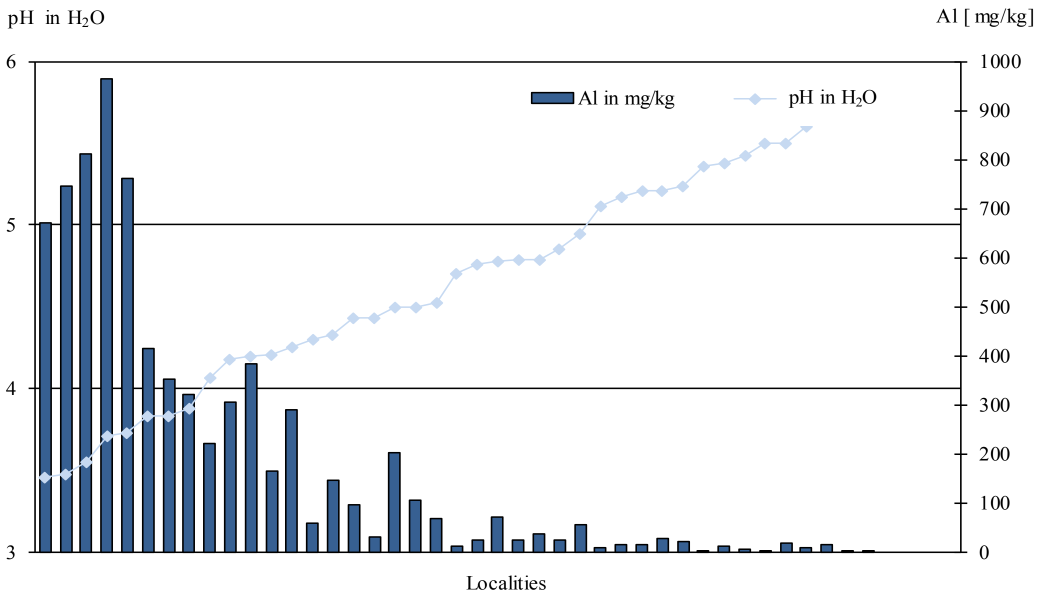


Figure 6. Active aluminium content in correlation with pH value

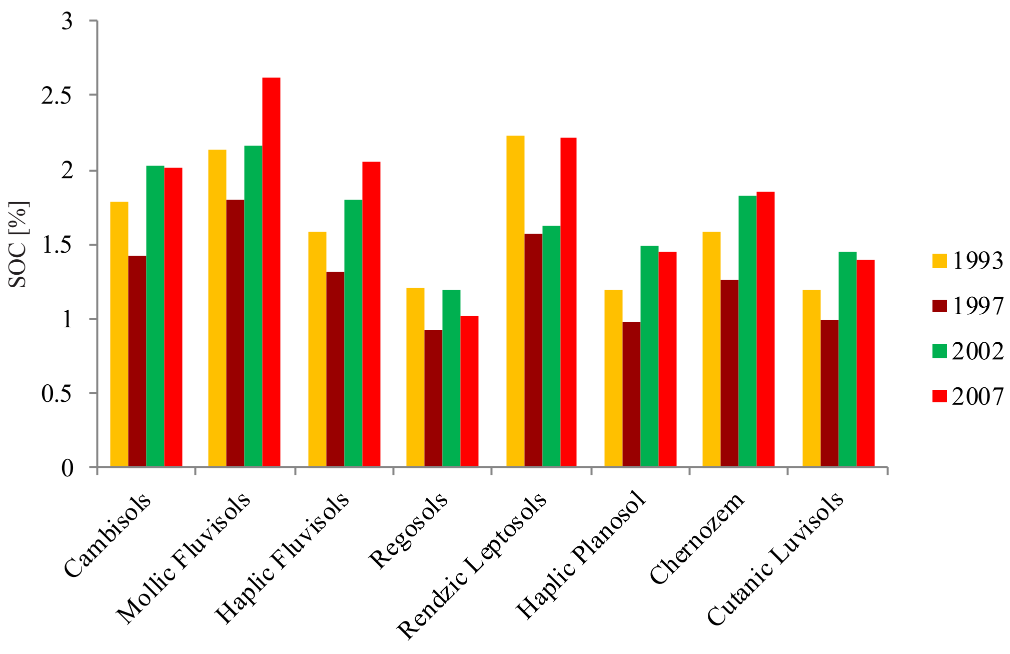


Figure 7. Comparison of soil organic carbon (SOC) [%] values in topsoil of arable soils of Slovakia

crete soil type as well as for the chemical structure of humic acids. These indicators seem to be a result of soil genesis and plant cultivation system, as well.

The content of organic carbon (SOC) in soils is largely conditional for the soil genesis and depends on soil use and soil management. The content of SOC in cultivated soils, mainly in arable soils, is limited by intensity and depth of cultivation what affect on increasing of mineralization. For this reason the average values of SOC in arable Slovak soils ranged from 1 to 2.5%. This corresponds low to high content of SOC (Hanes *et al.* 1995). The lowest values of soil organic carbon are typical for Regosols and the highest values of SOC for Phaeozems (FAO – WRB 2015).

Recently, development of the SOC content in arable soils is quite different depending on soil types. The gradual increase of SOC content is clear mainly in Phaeozems and Haplic Fluvisols. The average content of SOC in Rendzic Leptosols after previous decline substantially had increased to the level of initial state. SOC content in Chernozems and Cambisols are maintained at the same level as in the previous cycle. Slightly reduction of SOC content in Haplic Planosols and Luvisols was observed, but it was not statistically significant. The relatively highest reduction of SOC level was determined in Regosols. This soil type contains the lowest SOC level during whole monitoring period. Despite the fact that critical value of SOC is a function of soil texture, as well as climate, soil material and topographic position (Stockmann *et al*. 2013) we assume that SOC level of Regosols is below critical value of SOC.

Finally, the tendency to stabilization of soil humus content and its some increase has been observed during the last period after slight decline, especially in arable land since the beginning of soil monitoring in Slovakia. Changes in total nitrogen are consistent with SOC as confirmed by significant linear correlation between SOC an Nt content. In qualitative parameters of humus (C_{HA}/C_{FA}, Q^4_6) no significant changes were indicated during last 20 years (period of soil monitoring realization in Slovakia).

Soil contamination

The contaminants reduce soil fertility, change the species composition of the flora and fauna, and endanger human health due to the enrichment of heavy metals in the food chain (Maxwell 1991; Lepp *et al*. 1996; Dudka & Adriano 1997). During soil monitoring process was changed the hygienic legislative in Slovakia. Instead of extraction with 2M HNO_3 was introduced extraction with aqua regia with new hygienic limits (MPRV SR 2013). Therefore the latest distribution of risk elements (extracted with aqua regia) in Slovak agricultural soils is presented in the following Table 2.

Measured average values of risk elements in soils are lower than valid hygienic limit for Slovakia (MP SR 2004; MPRV SR 2013). The distribution of risk elements depends on parent material, land use, soil type and potential source of elements origin (geogenic, anthropogenic, resp. mixed influence) (Wilcke *et al*. 2005). Concerning the measured values of risk elements presented in the Table 2 the slightly increased values of some risk elements (Cd, Cu, Pb, Zn) from among the evaluated soil types occur on the Fluvisols which could be transported from the catchments and accumulated on the alluvial deposits especially along down the rivers (Kobza *et al*. 2014).

Increased values of some elements are also characteristic for some Cambisols which are particularly influenced by geochemical anomalies occurrence especially on crystalline rocks and volcanic deposits, as well. The most extended areas of geochemical anomalies appear in Štiavnické vrchy, Low Tatras and Slovenské rudohorie mountains. These regions are characterized by high and very high concentrations of risk trace elements, especially heavy metals in all soil profile (Cd, Pb, Cu, Zn, As and Hg) (Kobza & Gašová 2014). Therefore also mean value of some risk elements on Cambisols are also increased (As, Cu, Zn, Pb, Cr, Co) – Table 2. On the contrary, the soils with low to very low content of humus and clay fraction (Regosols) are characteristic with the lowest content practically of all risk elements, what it was already mentioned in some previous works (Linkeš *et al*. 1997; Wilcke *et al*. 2005).

Finally, significant change in concentration of risk elements (Cd, Pb, Cu, Zn, Cr, Ni, Co, Se, As) was not indicated during monitored period of 20 years in Slovakia (Kobza *et al.* 2014). It means that the soils which were contaminated at the beginning of soil monitoring process, are still contaminated at present. In addition, antropogenically deposited heavy metals are less strongly bound in soils, because chemical equilibration of heavy metals in soils is a long-term process (Chlopecka *et al*. 1996; Wilcke & Kaupenjohann 1997).

The obtained results concerning current soil degradation processes in Slovakia are briefly presented in the Table 3.

Negative changes were indicated at soil erosion (on Cambisols and Rendzic Leptosols), soil compaction (heavy Luvisols), soil acidification (acid Fluvisols, Albic Luvisols and Planosols, Cambisols and Podzols), decline in soil organic matter (Luvisols, Albic Luvisols, Planosols and Regosols – there are the soils with low content of soil organic matter), contamination (the highest values were determined on Fluvisols as a result of transport and accumulation of risk elements on fluvial deposits along the rivers and Cambisols often with occurrence of geochemical anomalies, resp. with their influence).

T a b l e 2

Average content of risk elements [mg/kg] extracted with aqua regia in arable layer (0–10 cm) of agricultural soils of Slovakia

Soils	As	Cd	Co	Cr	Cu	Ni	Pb	Zn	Se
FM	10.8	0.7	8.8	39.1	34.0	37.0	54.3	122.8	–
ČA	10.0	0.4	7.8	42.9	22.7	29.6	21.1	75.6	0.2
ČM	10.0	0.4	7.8	42.9	22.7	29.6	21.1	75.6	0.3
HM	9.2	0.2	10.0	41.5	22.9	32.6	19.7	68.8	0.1
LM+PG	9.9	0.3	9.7	42.8	17.0	23.3	24.2	66.7	0.2
KM	14.8	0.3	12.6	52.2	28.9	29.2	27.0	93.5	–
RM	3.4	0.1	2.0	19.5	17.0	12.0	7.7	41.0	0.3
RA	13.1	0.5	11.8	55.2	30.6	42.0	36.3	103.1	–

Explanations (soils according to FAO – WRB 2015): FM – Fluvisols, ČA – Phaeozems, ČM – Chernozems, HM – Luvisols, LM+PG – Albic Luvisols and Planosols, KM – Cambisols, RM – Regosols, RA – Rendzic Leptosols

T a b l e 3

Current state and development of soil degradation processes in Slovakia

Soils	Soil degradation processes				
	Erosion	Compaction	Acidification	Decline in soil organic matter	Contamination
FM	–	↑	↓	↑	↓
ČA	–	↑	–	↑	–
ČM	–	↑	–	↑	–
HM	–	↓*	–	↓	–
LM+PG	–	↑	↓	↓	–
KM	↓	↑	↓	–	↓
RM	–	–	–	↓	–
RA	↓	–	–	↑	–
PZ	–	–	↓	↑	–

Explanations (soils according to FAO – WRB 2015): FM – Fluvisols, ČA – Phaeozems, ČM – Chernozems, HM – Luvisols (*except heavy Luvisol), LM+PG – Albic Luvisols and Planosols, KM – Cambisols, RM – Regosols, RA – Rendzic Leptosols, ↑ – positive changes, ↓ – negative changes, – without significant change

In the contrast, the positive changes were indicated at soil compaction on some soil types (Fluvisols, Phaeozems, Chernozems, Albic Luvisols, Planosols and Cambisols), it may be said that the parameters of soil compaction are relatively dynamic and depend on way of agrotechnics. Especially during last period the stabilization of soil organic matter is observed (on Fluvisols, Phaeozems, Chernozems, Rendzic Leptosols and Podzols, which are originally rich in soil organic matter content).

CONCLUSIONS

On the basis of obtained results the most significant negative changes of agricultural land degradation processes have been recorded in soil erosion and particularly in soil acidification especially on acid rocks and contamination especially as a result of soil-sedimentary material transport and its accumulation on down the rivers deposits and geochemical anomalies influence, as well. The physical degradation was especially manifested in compacted and the eroded soils. It was calculated that about 39% of the agricultural land (mostly on Cambisols and Rendzic Leptosols) is potentially affected by soil erosion in Slovakia. The slight sensitivity to compaction was detected mostly on cultivated arable soils − texturally heavy Fluvisols, Chernozems, Luvisols, Albic Luvisols and Planosols and heavy Cambisols. It is necessary in the future pay attention to the arable land with pH values in slight acid and the acidic range as well as with a low quantity and quality of soil organic matter. To overcome the limitations of Al phytotoxicity, Ca amendments are an agronomic practice commonly used to reduce acidity effect on plant growth and development. The tendency to stabilization of soil humus content and its some increase has been observed during the last period after slight decline, especially in arable land since the beginning of soil monitoring in Slovakia. Significant change in concentration of risk elements was not determined during monitored period of 20 years. Slight increase of contaminants was determined especially on Fluvisols situated down the rivers and on some Cambisols mostly influenced by geochemical anomalies.

Acknowledgements. We would like kindly to thank to Ministry of Agriculture and Rural Development of the Slovak Republic for the financial support of this project No 471/2014-310/MPRV SR.

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Received: April 4, 2017

DE GRUYTER OPEN

Agriculture (Poľnohospodárstvo), 63, 2017 (2): 86−91

Short Communication DOI: 10.1515/agri-2017-0008

CRITERIA FOR ABNORMALITY EVALUATION OF SELECTED WEATHER PARAMETERS IN THE SLOVAK REPUBLIC

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DANILOVIČ, M. – HLAVATÁ, H. – ŠOLTYSOVÁ, B.: Criteria for abnormality evaluation of selected weather parameters in the Slovak Republic. Agriculture (Poľnohospodárstvo), vol. *63*, 2017, no. 2, p. 86–91.

The paper describes the procedure of calculation and assessment of deviations of the average air temperature from the normal (in relation to the normal 1961–1990) or long-term average and the percentage of normal precipitation or long-term sum of precipitation, valid for the Slovak Republic. Three evaluation tables clearly indicate both threshold limit values, which facilitate the classification of the calculated indices for air temperature and precipitation. Criteria presented in this work are fully applicable for weather conditions evaluation during the growing season of cultivated plants in the Slovak Republic.

Key words: air temperature, precipitation, normality, evaluation worksheet, evaluative criteria

Evaluation of weather conditions during the growing season of cultivated plants, during the year or during the various months of the year has been an essential part of research papers, students' theses, scientific and technical works, projects, vegetation tests and creation of growth models. The most commonly measured weather parameters are air temperature and precipitation, and they are frequently evaluated at many kinds of scientific and technical works, especially in agricultural and natural sciences.

Climate data are more useful when they are compared with normal values (WMO 2007). Climate normals, as discussed by the World Meteorological Organization, are not only used as predictors of future climate conditions, but are also used to provide a reference value for the computation of climate anomalies. A climatological normal is defined as the arithmetic average of a climate element (e.g., temperature) over a 30-year period. The current climate normal period is calculated from 1st January 1961 to 31st December 1990 and will be used until 2020 (WMO 1989). Climate normals valid for the territory of Slovakia are calculated and published by the Slovak Hydrometeorological Institute (Mikulová *et al.* 2015 a,b).

Calculated deviation from a normal value was the simplest way to express the relationship between observed and normal conditions and is widely used to detect climate changes. In meteorology, it is a very common way to approach drought or warmth situation by generating an index using meteorological data. These indices can be calculated for a variety of time scales. Usually, these time scales range from a single month to a group of months. For climate data comparison with the normal values, it is necessary

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to process them using evaluative criteria calculated for the observed territory. There are some differences within the processing data of air temperature and data of precipitation. The aim of this work is to make the evaluation criteria available and provide missing guidance for air temperature and precipitation data processing during the growing season of the cultivated plants in Slovak republic, in relation to the climate normal 1961–1990.

Air temperature

In cases when concurrent values of monthly or annual air temperature average are compared with normal, the difference tends to be constant (index). Deviation from a normal value is calculated as a difference between the valuated average air temperature and normal for identical period (e.g., month, year).The index is calculated as:

Table 1

Class limits [°C] for evaluating the air temperature abnormality of months, years, half years and winter months in the Slovak Republic

Month / period	Normal						
	extraordinary below normal[1]	very below normal[2]	below normal[3]	normal[4]	above normal[5]	very above normal[6]	extraordinary above normal[7]
	Long-term average						
	extraordinary cold[8]	very cold[9]	cold[10]	normal[11]	warm[12]	very warm[13]	extraordinary warm[14]
I.	< –6.5	–6.5 to –4.1	–4.0 to –2.1	–2.0 to 2.0	2.1 to 3.5	3.6 to 5.0	> 5.0
II.	< –7.0	–7.0 to –4.1	–4.0 to –1.6	–1.5 to 2.0	2.1 to 3.0	3.1 to 5.0	> 5.0
III.	< –5.0	–5.0 to –3.6	–3.5 to –2.1	–2.0 to 1.5	1.6 to 3.0	3.1 to 4.5	> 4.5
IV.	< –4.0	–4.0 to –2.6	–2.5 to –1.1	–1.0 to 1.0	1.1 to 2.5	2.6 to 4.0	> 4.0
V.	< –3.0	–3.0 to –2.1	–2.0 to –1.6	–1.5 to 1.5	1.6 to 2.5	2.6 to 3.5	> 3.5
VI.	< –2.5	–2.5 to –1.6	–1.5 to –1.1	–1.0 to 1.0	1.1 to 2.0	2.1 to 3.0	> 3.0
VII.	< –2.0	–2.0 to –1.6	–1.5 to –1.1	–1.0 to 1.0	1.1 to 1.5	1.6 to 2.5	> 2.5
VIII.	< –2.5	–2.5 to –1.6	–1.5 to –1.1	–1.0 to 1.0	1.1 to 1.5	1.6 to 2.5	> 2.5
IX.	< –3.0	–3.0 to –2.1	–2.0 to –1.1	–1.0 to 1.0	1.1 to 2.0	2.1 to 3.5	> 3.5
X.	< –3.5	–3.5 to –2.1	–2.0 to –1.1	–1.0 to 1.0	1.1 to 2.0	2.1 to 3.5	> 3.5
XI.	< –4.0	–4.0 to –2.1	–2.0 to –1.1	–1.0 to 1.0	1.1 to 2.0	2.1 to 3.5	> 3.5
XII.	< –5.5	–5.5 to –3.1	–3.0 to –1.6	–1.5 to 1.5	1.6 to 3.0	3.1 to 4.5	> 4.5
I.–XII.	< –1.5	–1.5 to –1.1	–1.0 to –0.6	–0.5 to 0.5	0.6 to 1.0	1.1 to 1.5	> 1.5
IV.–IX.	< –1.5	–1.5 to –1.1	–1.0 to –0.6	–0.5 to 0.5	0.6 to 1.0	1.1 to 1.5	> 1.5
X.–III.	< –3.5	–3.5 to –2.1	–2.0 to –1.1	–1.0 to 1.0	1.1 to 1.5	1.6 to 2.5	> 2.5
XII.–II.	< –5.0	–5.0 to –3.1	–3.0 to –1.1	–1.0 to 1.5	1.6 to 2.0	2.1 to 3.0	> 3.0

IV.–IX. – warm half-year; X.–III. – cold half-year; XII.–II. – winter;
Note that in Slovak language we never use term ‘extrémne’ and classes translate as: [1]mimoriadne podnormálny, [2]silne podnormálny, [3]podnormálny, [4]normálny, [5]nadnormálny, [6]silne nadnormálny, [7]mimoriadne nadnormálny, [8]mimoriadne studený, [9]veľmi studený, [10]studený, [11]normálny, [12]teplý, [13]veľmi teplý, [14]mimoriadne teplý

$$T - n(T)$$

where:
T denotes the longer period's average air temperature, *n(T)* is air temperature normal for the same period and valid for the evaluated site of Slovak Republic (Lapin *et al.* 1987).

Class limits for the calculated temperature difference categories are presented in the evaluation worksheet (Table 1). Presented class limits were elaborated following the methodology of Slovak Hydrometeorological Institute (Lapin *et al.* 1987) and are valid for the territory of Slovakia in relation to the normal 1961–1990. Each class has a verbal designation of abnormality level. If data are compared with normal, it is allowed to use just verbal designation for normal.

Evaluative criteria can be used if the air temperature deviation was calculated from long-term average (note that it must be clearly indicated for which period the long-term average was calculated; e.g. 1951–1980). In this case, the deviation is calcu-

Table 2

The index classes [%] for evaluating the precipitation abnormality of months, half years and years in the Slovak Republic, Zone I.

Month / period	Normal						
	extraordinary below normal[1]	very below normal[2]	below normal[3]	normal[4]	above normal[5]	very above normal[6]	extraordinary above normal[7]
	Long-term average						
	extraordinary dry[8]	very dry[9]	dry[10]	normal[11]	wet[12]	very wet[13]	extraordinary wet[14]
I.	< 20	20 to 39	40 to 59	60 to 130	131 to 170	171 to 230	> 230
II.	< 20	20 to 39	40 to 59	60 to 130	131 to 180	181 to 240	> 240
III.	< 20	20 to 39	40 to 59	60 to 130	131 to 180	181 to 250	> 250
IV.	< 30	30 to 49	50 to 69	70 to120	121 to 160	161 to 200	> 200
V.	< 30	30 to 49	50 to 69	70 to 120	121 to 160	161 to 210	> 210
VI.	< 30	30 to 49	50 to 69	70 to 130	131 to 160	161 to 210	> 210
VII.	< 20	20 to 49	50 to 69	70 to 130	131 to 170	171 to 220	> 220
VIII.	< 30	30 to 49	50 to 69	70 to 120	121 to 160	161 to 210	> 210
IX.	< 20	20 to 39	40 to 59	60 to 130	131 to 170	171 to 230	> 230
X.	< 0	0 to 29	30 to 59	60 to 130	131 to 170	171 to 230	> 230
XI.	< 10	10 to 29	30 to 59	60 to 130	131 to 180	181 to 240	> 240
XII.	< 20	20 to 39	40 to 69	70 to 130	131 to 170	171 to 220	> 220
I.–XII.	< 60	60 to 79	80 to 89	90 to 110	111 to 120	121 to 140	> 140
IV.–IX.	< 60	60 to 69	70 to 79	80 to 120	121 to 130	131 to 150	> 150
X.–III.	< 50	50 to 69	70 to 79	80 to 120	121 to 130	131 to 160	> 160

IV.–IX. – warm half-year; X.–III. – cold half-year;
Note that in Slovak language we never use term 'extrémne' and classes translate as: [1]mimoriadne podnormálny, [2]silne podnormálny, [3]podnormálny, [4]normálny, [5]nadnormálny, [6]silne nadnormálny, [7]mimoriadne nadnormálny, [8]mimoriadne suchý, [9]veľmi suchý, [10]suchý, [11]normálny, [12]vlhký, [13]veľmi vlhký, [14]mimoriadne vlhký

lated as a difference between the valuated average air temperature and long-term average for identical period (month, year), valid for the evaluated site of Slovak Republic. The index is calculated as:

$$T - l(T)$$

where:
T denotes the longer period's average air temperature, *l(T)* is long-term average for the same period and valid for the evaluated site of Slovak Republic (Lapin *et al.* 1987).

If data are compared with long-term average, it is allowed to use verbal designation for normal and long-term average as well.

Precipitation

Drought is considered as one of the biggest natural disasters that affects the society more than others. Drought as well as wet can be computed by using quantitative indices in the time scale. Indices measure how much precipitation for a given period of time has deviated from historically established

T a b l e 3

The index classes [%] for evaluating the precipitation abnormality of months, half years and years in the Slovak Republic, Zone II.

Month / period	Normal						
	extraordinary below normal[1]	very below normal[2]	below normal[3]	normal[4]	above normal[5]	very above normal[6]	extraordinary above normal[7]
	Long-term average						
	extraordinary dry[8]	very dry[9]	dry[10]	normal[11]	wet[12]	very wet[13]	extraordinary wet[14]
I.	< 10	10 to 29	30 to 49	50 to 140	141 to 190	191 to 280	> 280
II.	< 0	0 to 29	30 to 59	60 to 140	141 to 190	191 to 260	> 260
III.	< 10	10 to 29	30 to 49	50 to 130	131 to 190	191 to 280	> 280
IV.	< 10	10 to 39	40 to 59	60 to 130	131 to 170	171 to 210	> 210
V.	< 10	10 to 39	40 to 59	60 to 130	131 to 160	161 to 220	> 220
VI.	< 10	10 to 39	40 to 69	70 to 140	141 to 180	181 to 250	> 250
VII.	< 10	10 to 39	40 to 59	60 to 140	141 to 180	181 to 260	> 260
VIII.	< 20	20 to 39	40 to 59	60 to 130	131 to 170	171 to 250	> 250
IX.	< 0	0 to 19	20 to 49	50 to 130	131 to 180	181 to 260	> 260
X.	< 0	0 to 19	20 to 49	50 to 130	131 to 190	191 to 280	> 280
XI.	< 0	0 to 29	30 to 59	60 to 140	141 to 180	181 to 250	> 250
XII.	< 10	10 to 39	40 to 69	70 to 140	141 to 180	181 to 230	> 230
I.–XII.	< 60	60 to 79	80 to 89	90 to 110	111 to 120	121 to 140	> 140
IV.–IX.	< 60	60 to 69	70 to 79	80 to 120	121 to 130	131 to 150	> 150
X.–III.	< 50	50 to 69	70 to 79	80 to 120	121 to 130	131 to 160	> 160

IV.–IX. – warm half-year; X.–III. – cold half-year;
Note that in Slovak language we never use term 'extrémne' and classes translate as: [1]mimoriadne podnormálny, [2]silne podnormálny, [3]podnormálny, [4]normálny, [5]nadnormálny, [6]silne nadnormálny, [7]mimoriadne nadnormálny, [8]mimoriadne suchý, [9]veľmi suchý, [10]suchý, [11]normálny, [12]vlhký, [13]veľmi vlhký, [14]mimoriadne vlhký

norms. Any forms of drought and wet are related to some antecedent and relative precipitation amounts for the previous period. The simplest expression of difference from the normal could be defined as a drought index, the so called precipitation index percent of normal:

$$P / n(P) \times 100$$

where:
P denotes the longer period's sum of precipitation,
n(P) is precipitation normal for the same period and valid for the evaluated site of Slovak Republic (Lapin *et al.* 1987).

Table 2 and 3 shows the precipitation index thresholds elaborated following the methodology of Slovak Hydrometeorological Institute (Lapin *et al.* 1987) valid for the territory of Slovakia in relation to the normal 1961–1990.

In case of annual and half-year precipitation, the evaluation index thresholds are the same in both tables and are valid for all sites in Slovakia.

To rate the monthly precipitation abnormality evaluation, it is necessary to see and use the Figure 1. Evaluated site (site belongs to evaluated precipitation data) must be integrated into the zone in relation to the evaluated period of year using the map presented in Figure 1. The territory of Slovakia is divided into two zones (Zone I and Zone II) for each period of the year, and the year is divided into four periods (three months in period). There are two periods of the year (February to April and August to October) in the map with the same zoning. Table 2 is valid for the sites integrated to Zone I and Table 3 is valid for sites integrated to Zone II.

Verbal designation of abnormality levels is presented in Table 2 and 3 together with the mentioned precipitation index thresholds. If data are compared with normal, it is allowed to use just verbal designation for normal.

Evaluative criteria can be used if precipitation index is calculated from long-term sum (note that the period of long-term average calculation must be clearly indicated; e.g., 1951–1980). In this case, the precipitation index percent of normal is calculated as:

$$P / l(P) \times 100$$

where:
P denotes the longer period's sum of precipitation,
l(P) is the precipitation long-term average for the same period and valid for the evaluated site of Slovak Republic (Lapin *et al.* 1987).

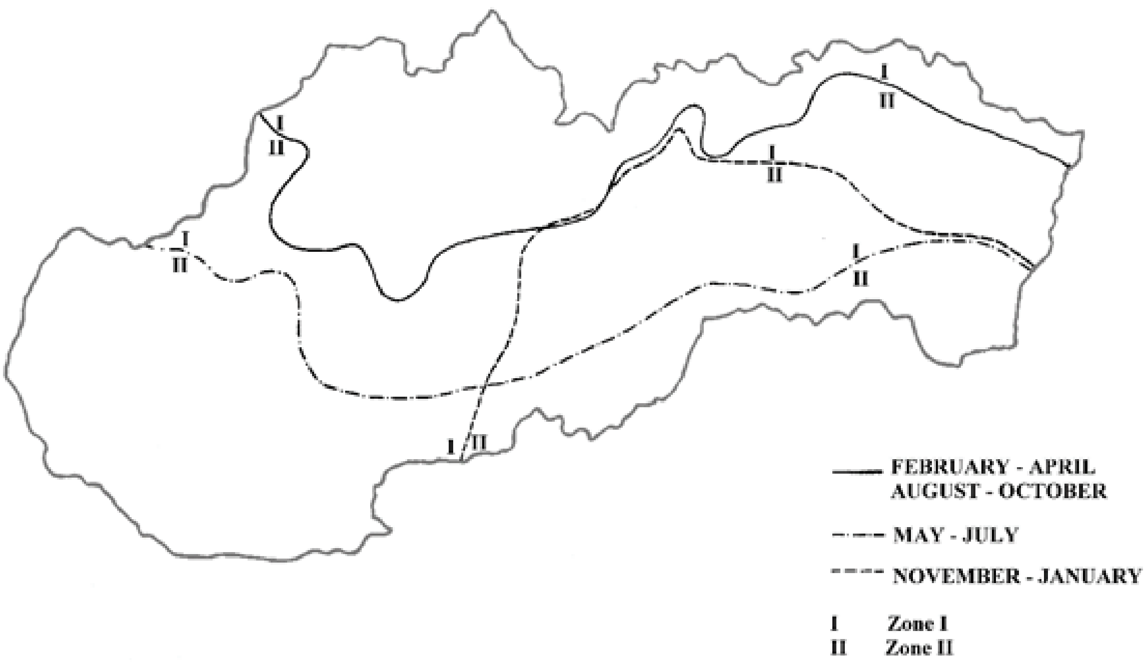

Figure 1. Division of territory of Slovak Republic into Zone Land II based on the amount of precipitation over a period of three months (Lapin *et al.* 1987)

Next step in the procedure is the same as for comparing with normal, in case of monthly precipitation evaluation, the site must be integrated in to one of two zones (see above). If data are compared with long-term sum, it is allowed to use verbal designation for normal and for long-term sum as well.

CONCLUSIONS

Air temperature and precipitation data are more useful when they are compared with normal values or long-term values. Relationship between observed and baseline conditions is simply expressed by climate index. The purpose of the index is to reduce complex conditions to a single number that retains some physical meaning and can be used to monitor a particular process. Climate indices are widely used to characterise features of the climate for climate prediction and to detect climate change. They may apply to individual climatological stations or describe some aspect of climate of the area. Climate indices must be calculated using the relevant data of normal or long-term averages and relevant evaluative criteria. The criteria presented in this work are fully applicable for weather evaluation in the Slovak Republic.

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Received: February 2, 2017