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ORIGINAL PAPER

Nickel dynamics influenced by municipal solid waste compost application in tea (*Camellia sinensis* L.): a cup that cheers

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Abstract Effects of municipal solid waste compost application on tea (*Camellia sinensis* L.) cultivation (Tocklai Vegetative clone 1 and Tocklai Vegetative clone 23) was studied with respect to biomass yield, soil nickel risk, nickel uptake and transfer to tea infusion. Application of municipal solid waste compost @ 2–6 t ha^{-1} in soil lowered the risk assessment code of nickel by increasing non-labile nickel pool. Reduced Ni translocation factor from root to stem to leaf led to low nickel accumulation in leaf indicating high nickel tolerance ability of tea. Tea infusions from Tocklai Vegetative clone 1 and Tocklai Vegetative clone 23 with municipal solid waste compost application in soil up to 10 t ha^{-1} showed leaf nickel contents below permissible limit, i.e., from 0.002 to 1.2 and 0.01 to 1.1 μg L^{-1}, respectively. Municipal solid waste compost could therefore be a valuable alternative for soil amendment subject to non-enhancement of soil nickel storage on long-term use. The one-way analysis of variance along with Duncan's multiple range tests showed significant differences between pair of treatments. Hierarchical cluster analysis revealed formation of three different groups between the clones and treatments imposed.

Keywords Bioaccumulation factor · Risk assessment code · Tea infusion · Tocklai Vegetative clone · Translocation · Tolerance index · Yield

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Introduction

Tea (*Camellia sinensis* L.) is a perennial (continues the effective production up to 70 years) and acidophilic crop, which grows well in tropical and subtropical regions (45°N–34°S) of 45 countries spread over all the continents except North America (Karak and Bhagat 2010). Tea is known as widely consumed non-alcoholic, cheaper and stimulating ancient beverage with several health benefits (Karak et al. 2011). Among the tea producing countries, India is the second largest tea producing one and Assam tea (Assam, a state in northeast India contributing about 56 % of total Indian tea production) is famous in the global market for its quality (Tea statistics of India 2013). However, declining yield of Assam tea production could be related to the degradation of soil health due to the intensive agricultural practices, indiscriminate use of chemical fertilizers and cultivation of high-yielding tea crop. Therefore, it is of paramount importance to manage the soil health by adding organic materials in soil as most of the tea growing soil in northeast India (total cultivated tea land in Assam, a well-known tea growing belt in northeast India, is around 322.21 thousand ha in the year 2011 as reported by Tea Board of India; Tea statistics of India 2013) is categorized as soil having low organic carbon content (<1 %). This low organic carbon status in tea soil invites the need for proper management of organic carbon in soil through addition of compost, which is a common practice in most of the tea gardens. However, because of scarcity of commonly used organic materials like cow dung, agricultural waste and so forth, alternative composting materials need to be found out. It has been documented that municipal solid waste (MSW) could be one of the alternative sources of composting materials as it is available not only free of cost but also beneficial toward environmentally sound disposal and resource recovery. Presently, Indian cities generate about 32 million tons of non-segregated MSW annually, but it is supposed to increase to 299.3 million tons by the year 2047 (Karak et al. 2012). A new generation of organic soil amendments has appeared due to the composting of these wastes. The use of MSW compost (MSWC) as a manure is attractive but may pose an environmental risk due to its metal content and to the presence of other pollutants (Carbonell et al. 2011). Several studies highlighted the beneficial effects of MSWC application in soil as well as growing crops as it increases major nutrients, bioavailable macronutrients, total soil porosity, water penetration, air circulation and increased water retention in soil as well as improves the stability of soil aggregates and yield of crop in impoverished soil (Weber et al. 2007). Pervasive adequacy of heavy metals (HMs) could adversely affect soil fertility, which constitute a long-term environmental hazard due to the high residence time in soil and leads to its subsequent contamination in the food chain (Achiba et al. 2009). Thus, it is highly important to judge the beneficial aspects of MSWC along with the potentially detrimental ones (Weber et al. 2007). Among the several HMs in MSWC, nickel (Ni) could have detrimental effect both in soil and to plants. The adverse effect of Ni in the environment has been reported due to its long persistence. Toxic levels of Ni in plants range from 8 to 147 mg kg^{-1} (Gupta et al. 2008) even though Ni is an essential micronutrient at very low contents (<50 µg g^{-1} in the plant tissue) for plant growth (Gupta et al. 2008). The essentiality of Ni in plant is established by the documentation of various Ni deficiency symptoms as well as its physiological functions and critical roles in various enzymes in plants (Sreekanth et al. 2013). Besides several beneficial roles of Ni in plant, it has been documented that Ni toxicity of plant grown in soil amended with large quantities of wastes, e.g., sewage and sludge (Gupta et al. 2008). The symptoms of Ni toxicity reported in plants are the inhibition of growth, chlorosis, necrosis and wilting (Sreekanth et al. 2013). High concentrations of Ni may contribute to deficiency of nutrients particularly divalent cations that come in competition with Ni. Excess Ni has been shown to cause a substantial decrease in all macro- and micronutrients in leaves of different plants. Sreekanth et al. (2013) also reported that for higher plants, Ni strongly influences metabolic reactions and has the capability to form reactive oxygen species which may cause oxidative stress. However, there is no information available for Ni in tea plant.

After a critical survey of the available literature, it has been seen that most of the research outcomes are published on pseudo total heavy metals in soil including Ni, as it reflects the geological origins of soil as well as the anthropogenic inputs. However, determination of pseudo total Ni does not give accurate estimate of the likely environmental impact as the use of pseudo total Ni concentration as a criterion to assess the potential effects of soil contamination implies that all forms of a given element have an equal impact on the environment; such an assumption is clearly untenable (Karak et al. 2011; Tessier et al. 1979). Therefore, fractionation of Ni in soil using sequential techniques either through Tessier technique (Tessier et al. 1979) or through European Community Bureau of Reference (BCR) procedure is an important tool for chemical characterization that can provide useful information on its potential mobility, availability and assimilation by various parts of plants (Achiba et al. 2009). Sequential extraction of Ni makes a possibility to gain a greater insight into identifying how Ni is bound, indicating the MSWC toxicity (Mäkelä et al. 2013; Rajapaksha et al. 2012).

Fractionation study on Ni also provides a classification of soil, according to element mobility, through the risk assessment code (RAC). The RAC assesses the potential release of elements by the percentages of water-soluble, exchangeable, and carbonate-bound fractions are obtained following Tessier's sequential extraction scheme) in soil (Singh et al. 2005). Therefore, RAC may be useful to quantify potential environmental effects and as reliable indicator of health of the ecosystem (Singh et al. 2005). Besides RAC, bioaccumulation factor (BAF: ratio between metal concentration in plant tissue and metal concentration in soil), the soil-to-plant transfer factor (TF: the ratio of the concentration of pollutant in plant tissues to its concentration into the root) and the tolerance index (T_i: dry matter yield of MSWC treated soil/dry matter yield of untreated soil) are often used to quantify the interactions between soil compartments and plants for metal of interest (Carbonell et al. 2011; Karak et al. 2015a).

To the best of our knowledge, several research efforts have been put forwarded in regard to Ni translocation from soil to the major common plants (or crops) where MSWC has been applied as organic amendments (Achiba et al. 2009; Carbonell et al. 2011; Weber et al. 2007; Zheljazkov and Warman 2004). While beneficial role of MSWC and HMs dynamics in different crops amended with MSWC is known, the effect of MSWC for tea plantation is very limited and no information is available in the literature as well. Thus, in the present experiment we have aimed at providing the insights on possible role of MSWC on tea plantation through a pot experiment in light of Ni dynamics in soil–plant system and in turn on the crop yield and Ni in tea infusion. It is done to judge the suitability of MSWC compost as an alternative source of organic amendment for tea cultivation as there has been too much speculation on the toxic effect of MSWC through Ni pollution in tea plantation. The experiment was started in May 2011 and completed in April 2013 at experimental site of Upper

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Materials and methods

Chemical reagents

All the chemical reagents and standard solutions of cadmium (Cd), chromium (Cr), copper (Cu), Ni and zinc (Zn) used for this experiment were of analytical grade and were purchased from Merck India Ltd (Mumbai).

Soil sample used for experiment

Sixteen soil samples (0–15 cm depth) from different locations were collected from standing tea area of Tocklai Experimental firm (26°45′N, 94°13′E), Assam, India. Samples were left in the field for natural drying until constant weight. Natural drying of soil samples was done instead of oven drying as oven drying of soil samples can lead to some alteration in the different fractions of metals of interest in soil matrixes (Topp and Ferré 2002). All the samples were mixed uniformly to produce a representative sample. A small quantity from the mixture was further dried naturally under shade, sieved through 2-mm sieve and kept in an air tight plastic jar at room temperature.

Compost preparation

Municipal solid waste (MSW) samples were collected by crap sampling method and were segregated manually. Samples were collected during winter in the year 2010. Segregated MSW samples were chopped by a chopper to 1–2 cm particle size to increase the reacting surface and obtain better aeration and moisture control (Karak et al. 2014a). Composting was performed, both in summer and in winter seasons in concrete pits with three replicates. Samples (about 4 kg) were taken from five symmetrical locations of each of the concrete pits, at the end of the composting process (56th day). Compost samples were air-dried and then passed through 1-mm sieve for analysis (FCOI 1985).

Greenhouse studies and experimental design

One-and-half-year-old nursery plants of TV1 (Tocklai Vegetative 1; this clone is known as quality clone) and TV23 (Tocklai Vegetative 23; this clone is known as yield clone) clones having identical root collar diameter (the diameter of the main stem measured at 2 cm from the root collar) were selected as test cultivars for pot experiment. TV1 and TV23 clone were selected as both are commonly used cultivars in tea estates for commercial tea production. Earthen pots were used with 77 cm upper diameter, 41 cm bottom diameter and 16 cm height for pot experiment. Pots were filled up with exactly 10 kg soil, basal dose of N (90 kg N ha^{-1} as urea), K (90 kg K_2O ha^{-1} as potassium chloride) and 50 g of P (1.48 % P as P_2O_5)-enriched vermin-compost was mixed (except control treatment) thoroughly in each pot (Barooah et al. 2005). P-enriched vermin-compost was collected from Tocklai vermin-compost unit. Preparation procedure and its physical and chemical properties are available in the report by Bisen et al. (2011). Addition of vermin-compost in pit mixture is a common agricultural practice in tea planting to ensure supply of nutrients to young plant (Karak et al. 2014a). We did not compare MSWC with commercially available compost in the present study as addition of commercial compost in tea industry is not frequently observed. Furthermore, we formulated this experiment in a way that resembles the common practices of tea estate where composts are prepared locally for their own use as far as possible. Altogether six treatments were imposed for present investigations, viz. T0: control (without NPK); T1: 2 t ha^{-1} MSWC; T2: 4 t ha^{-1} MSWC; T3: 6 t ha^{-1} MSWC; T4: 8 t ha^{-1} MSWC; and T5: 10 t ha^{-1} MSWC. All the treatments were imposed after a month of plantation. It was done to get the stable growth of plant. Soil samples were collected imposing 1 month of treatments to judge the changes in physical chemical properties of soil influenced by MSWC. All the pots were arranged by using complete randomized design (CRD) with three replications. The plants were then allowed to grow for 2 years (May 2011–April 2013). Experimental pots were kept in a greenhouse and were allowed to grow under at 62 % relative humidity. Pots were then maintained at 70 % field water holding capacity by adding Ni-free tube well water, at every 2- to 3-day interval. Soil moisture was measured by using tensiometer.

Soil sampling and pretreatment

Vermin-compost was used in all the treatment pots barring control one as a common basal dose considering least possibility of significant change and variation in physico-chemical properties within that short period of time. However, for each treatment, soil was sampled again after imposing the treatments (July 2011; referred as initial soil samples) and finally after 2 years from imposing the treatments (April 2013; referred as final soil samples). Soil samples were collected with a soil auger up to a depth of 10 cm. The 10 cm depth was chosen as it is considered as the depth for top soil. Three cores were taken for each treatment and were mixed to get a representative sample. Collected soil samples were then pretreated for analysis and preserved according to the protocol described by Karak et al. (2011).

Plant sampling and pretreatment

Plants were uprooted with the help of flowing tap water after 2 years of MSWC treatments. Plants were carefully rinsed with tap water to clean the soil and dust particles with it and then with deionized water for three times. The washed plants were parted into roots (feeder roots and lateral roots), stems and leaves. Plant samples were dried at 60 °C for 2 days to get constant weight and then roots, stems and leaves were weighed separately and the biomass was expressed as g $plant^{-1}$. The dried plant samples were then ground and homogenized using an agate pestle and then sieved through 40-mesh screen and finally stored in porcelain airtight stopper jars awaiting analysis.

Chemical analysis

To complete this experiment, several parameters, viz. pH, electrical conductivity (EC), sand, silt, clay, organic carbon, water-soluble carbon, cation exchange capacity (CEC), total N, P, K, Cd, Cr, Cu, Ni, and Zn; plant available Cd, Cr, Cu, Ni, and Zn; different fraction of Ni in soil and prepared compost were analyzed related to different studies aspect. Protocols described in FCOI (1985) were implemented in analyzing soil pH, EC and CEC.

Total organic carbon and water-soluble organic carbon in soils and MSWC were measured using the methodologies described by FCOI (1985). Total N contents in soils and MSWC were determined by Kjeldahl automatic analyzer following the procedure described by FCOI (1985). Sulfuric acid (H_2SO_4) digestion procedure (0.3 g of sample + 10 mL 98 % of H_2SO_4) was followed for total P analysis (FCOI 1985). Potassium was measured colorimetrically according to the FCOI (1985). Plant available Cd, Cr, Cu, Ni and Zn in soil as well as prepared MSWC was determined employing the outline described by Lindsay and Norvell (1978). In brief, 0.005 M diethylenetriaminepentaacetic acid (DTPA) solution was prepared by mixing 0.005 M DTPA, 0.01 M $CaCl_2$ and 0.1 M triethanolamine and the solution was adjusted to pH 7.30 ± 0.05. The initial DTPA solution was slightly acidic in nature, and pH was adjusted by using ammonium hydroxide. After that 5 g sample was mixed with 10 mL of DTPA solution, and the mixture was shaken for 2 h in an orbital shaker. The sample suspension was filtered through Whatman no. 41 filter paper and analyzed for Cd, Cr, Cu, Ni and Zn. However, pseudo total K, Cd, Cr, Cu, Ni and Zn were determined by aqua regia solution, consisting of HCl and HNO_3 in a 3:1 ratio (v/v). Pseudo total Ni from plant samples were also extracted using the same protocol. Ni fractionation in soils and prepared MSWC was performed based on the scheme of the work of Tessier et al. (1979).

Quality control and analysis of metals

Extracted heavy metals (Cd, Cr, Cu, Ni and Zn) concentrations were determined using flame Atomic Absorption Spectrophotometer (Mod. AA240, Agilent, Malaysia). To check the accuracy of analytical results and precision of the measurements as well as to validate the applied methods for the metal analysis in soil, compost, plants and tea infusion, the two standard reference materials, viz. SRM-2710: Montana soil and BCR-144: sewage sludge, were analyzed following the same digestion and analytical procedure as those of the samples. Recoveries from soil sample SRM-2710 were good with an average of 99.6 % for Cd (21.71 mg kg^{-1}), 104 % for Cr (40.56 mg kg^{-1}), 109.8 % for Cu (3245 mg kg^{-1}), 95.2 % for Ni (13.61 mg kg^{-1}) and 99.3 % for Zn (6903.34 mg kg^{-1}). BCR-144 also presented good recoveries with an average of 98.6 % for Cd (1.81 mg kg^{-1}), 101.5 % for Cr (91.35 mg kg^{-1}), 103.8 % for Cu (311.40 mg kg^{-1}), 99.6 % for Ni (44.72 mg kg^{-1}) and 101.2 % for Zn (930.03 mg kg^{-1}).

Phytotoxicity assay of prepared compost

Germination index (GI), also known as phytotoxicity test, was determined to judge the suitability of prepared compost for field application. The details of the methodologies used for this test will be found at Karak et al. (2014b). Phytotoxicity test was done on the basis of GI of wheat (*Triticum aestivum* L.; cv. PBW 3) and Indian mustard (*Brassica campestris* L.; cv. Pusa Jaikisan) seeds. Germination index (GI) was determined as follows:

$$\text{RSG}\,(\%) = \frac{\text{number of seeds germinated in soil amended with compost}}{\text{number of seeds germinated in soil without compost}} \times 100$$

$$\mathrm{RRG}\,(\%) = \frac{\text{mean root length in soil amended with compost}}{\text{mean root length in soil without compost}} \times 100$$

$$\mathrm{GI}\,(\%) = \frac{\mathrm{RSG} \times \mathrm{RRG}}{100}$$

where RSG is the percentage of relative seed germination and RRG is relative root growth.

Data analysis

Risk assessment code (RAC)

Risk assessment code (RAC) of Ni in soil was performed following the procedure described by Singh et al. (2005) as $\mathrm{RAC}\,(\%) = \left(\sum_{n=1}^{3} F_n \Big/ \sum_{n=1}^{6} F_n\right) \times 100$ where "F_n" is concentration of Ni in "nth" fraction.

Bioaccumulation factor (BAF)

A protocol described by Carbonell et al. (2011) was applied for BAF of Ni. In brief, BAFs were calculated as the ratio between the metal tissue concentration and the metal (total or available) concentration in soil.

$$\mathrm{BAF}_i = \frac{C_i}{C_{\mathrm{soil}}}$$

where C_i is Ni concentration in plant tissue (e.g., root, shoot and leaves) and C_{soil} is the Ni concentration in soil.

Transfer factor (TF)

The transfer factor (TF) of Ni in different parts of the tea plant samples has been applied according to the method of Carbonell et al. (2011):

$$\mathrm{TF} = \frac{C_{\text{aerial part}}}{C_{\text{root}}}$$

where $C_{\text{aerial part}}$ and C_{root} are Ni concentration in an aerial part of tea plant (viz. stem and leaves) and root of tea plant, respectively.

Tolerance index (T_i)

The tolerance index (T_i) was calculated following the procedure described by Karak et al. (2015a):

$$T_i = \text{Dry matter yield of MSWC tretaed soil/ Dry matter yield of untreated soil}$$

Preparation of tea leaf infusion and estimation of Ni

Tea leaves were separately collected from each pot, and tea leaf infusion was prepared following the procedure described by Karak et al. (2015a). In this study, the normal commercial process of tea manufacturing was not followed due to lack of sufficient amount of pluckable tea shoots in plants as experiment was conducted in pot. Tea infusion was prepared according to Indian traditional system. In brief, 2 g of dried young tea leaf shoot (afterwards referred as black tea) was boiled with 150 mL distilled water for 5 min with intermittent stirring to ensure the proper wetting of leaves and extraction of organic and inorganic matter (Karak and Bhagat 2010). The suspension was kept for another 10 min with a cover and large particles, and leaves were removed by a sieve. Thereafter, infusion was filtered through Whatman 1 filter paper and made up to 200 mL (which is equivalent to one cup of tea) in a volumetric flask. Ni in infusion was directly analyzed using Atomic Absorption Spectrophotometer (Mod. AA240, Agilent, Malaysia).

Statistical analysis

Pearson's correlation coefficients were computed separately for TV1 and TV23 between leave, stem, main root and feeder root biomass with all other variables. A one-way analysis of variance (ANOVA) was used to determine significant differences among treatments. A one-way ANOVA is an extension of the independent group t test where there are more than two groups. It considers one treatment factor with two or more treatment levels. The goal of the analysis is to test the differences among the means of the levels and to quantify these differences. It is assumed that the treatments are randomly assigned to different groups and the data within each group are normally distributed with equal variances across groups. Duncan's multiple range test (DMRT) was used to calculate the significant differences between means of treatment pairs. The application of Duncan's multiple range test is similar to that of the least significant difference (LSD) test. DMRT involves computation of numerical boundaries that allow for the classification of the difference between any two treatment means as significant or nonsignificant. DMRT requires computations of a series of values each corresponding to a specific set of pair comparisons unlike a single value for all pair-wise comparisons in case of LSD. It primarily depends on the standard error of the mean difference as in case of LSD. This can easily be worked out using the estimate of variance of an estimated elementary treatment contrast through the design.

Hierarchical clustering algorithm was applied in order to form homogenous groups among different treatments based on all other variables, viz. fractionation at different levels, RAC, biomass in main roots, feeder root, stem, leaves, and Ni content in main root, feeder root, stem and leaves.

Results and discussion

Characterization of soil and MSWC

Table 1 shows the basic physical and chemical properties of soil and the prepared MSWC. Experimental soil showed acidic pH (5.26 ± 0.05), which is usual in tea growing soil (Karak and Bhagat 2010; Karak et al. 2014a, 2015b, c). The prepared MSWC employed was alkaline whose pH was in line with the findings of Paradelo et al. (2011) where MSWC was produced from the source-separated organic fraction of MSW.

The slightly alkaline pH of prepared compost could be due to the characteristic of waste to neutralize the organic acid that was either generated from the anaerobic degradation of organic matter or might be influenced by the proteolysis occurring during the organic matter degradation (Karak et al. 2014a, 2014c, 2015a). MSWC compost had a much higher EC compared to the soil. Achiba et al. (2009) explained the high EC in MSWC due to the presence of soluble salts. The substantial quantity of total nitrogen

Table 1 Selected physical and chemical properties of used soil and prepared MSWC along with germination index (results are expressed on dry weight basis except germination index and unit is in mg kg^{-1} unless otherwise stated, values represent the mean of three replications ± SEM)

Parameters	Soil used for experiment	MSWC†
pH (unit less)	5.26 ± 0.05	7.46 ± 0.12 (6.5–7.5)
EC (dSm^{-1})	0.05 ± 0.01	3.36 ± 0.04 (≤4.0)
Organic carbon (%)	1.21 ± 0.08	21.40 ± 0.50 (>16.0)
Water-soluble carbon (%)	0.05 ± 0.02	0.73 ± 0.09
CEC (cmol kg^{-1})	8.26 ± 0.68	82.30 ± 1.90 (≥60)
Total N (%)	0.15 ± 0.03	1.56 ± 0.08 (≥0.5)
Total P as P_2O_5	9.26 ± 0.40	2.82 ± 0.14 (≥0.5)
Total K as K_2O	91.27 ± 8.28	14.90 ± 1.40 (≥1.0)
Total heavy metal:		
Cd	0.02 ± 0.01	2.47 ± 0.65 (5)
Cr	1.26 ± 0.04	8.97 ± 0.06 (50)
Cu	10.21 ± 1.08	62.58 ± 1.16 (300)
Ni	1.82 ± 0.03	8.22 ± 0.27 (50)
Zn	67.23 ± 2.55	288.47 ± 8.87 (1000)
DTPA extractable heavy metal:		
Cd	<0.002 ± –	0.67 ± 0.02
Cr	0.09 ± 0.02	1.04 ± 0.02
Cu	8.27 ± 0.06	9.82 ± 0.16
Ni	0.97 ± 0.02	1.14 ± 0.08
Zn	29.47 ± 0.72	62.43 ± 0.80
Different fraction of Ni*:		
F1	0.02 ± 0.001	0.18 ± 0.01
F2	0.01 ± 0.001	0.23 ± 0.01
F3	0.01 ± 0.001	0.98 ± 0.03
F4	0.61 ± 0.002	1.11 ± 0.04
F5	0.57 ± 0.001	3.94 ± 0.02
F6	0.16 ± 0.002	2.04 ± 0.01
Germination index (%)		
Wheat (*Triticum aestivum* L.)	Not done	96.00 ± 5.00
Indian mustard (*Brassica campestris* L.)	Not done	98.25 ± 5.00

* F1: water-soluble, F2: exchangeable, F3: bound to carbonates, F4: bound to Fe and Mn, F5: organically bound and F6: residual fractions; † values in parenthesis indicate the Legislation for MSWC addition in India(FCOI 1985)

(1.56 ± 0.08 %) highlights the benefit of using MSWC as an agricultural fertilizer. The pseudo total heavy metal content in the prepared MSWC is within the permissible limit for safe use in agricultural soil according to the Legislation for MSWC addition in India (FCOI 1985).

Selected physical and chemical properties of soil after receiving different doses of MSWC treatments are presented in Table 2. With respect to the control soil, all the MSWC-amended soil showed increased values of pH, organic C and major nutrient contents, whereas EC was not significantly changed. In general, all the physical and chemical properties of MSWC-treated soil significantly varied with the tea clones used. Karak et al. (2015c) reported significant increase in soil pH due to compost

Table 2 Selected physical and chemical properties of soil a month after receiving MSWC treatments (results are expressed on dry weight basis and unit is in mg kg^{-1} unless otherwise stated, values represent the mean of three replications ± SEM)†

Parameters	Tea clone	Treatments‡				
		T1	T2	T3	T4	T5
pH (unit less)	TV1	5.35 ± 0.02$^{\#a}$	5.38 ± 0.01^{a}	5.37 ± 0.03^{a}	5.41 ± 0.01^{a}	5.41 ± 0.04^{a}
	TV23	5.33 ± 0.01^{a}	5.36 ± 0.03^{a}	5.36 ± 0.02^{a}	5.39 ± 0.01^{a}	5.40 ± 0.02^{a}
EC (dSm^{-1})	TV1	0.07 ± 0.01^{a}	0.07 ± 0.02^{a}	0.08 ± 0.01^{a}	0.09 ± 0.01^{a}	0.09 ± 0.02^{a}
	TV23	0.07 ± 0.01^{a}	0.08 ± 0.01^{a}	0.10 ± 0.02^{a}	0.10 ± 0.03^{a}	0.13 ± 0.01^{a}
Organic carbon (%)	TV1	1.24 ± 0.01^{a}	1.27 ± 0.01^{a}	1.29 ± 0.02^{a}	1.31 ± 0.03^{a}	1.34 ± 0.04^{a}
	TV23	1.22 ± 0.01^{a}	1.25 ± 0.02^{a}	1.31 ± 0.03^{a}	1.33 ± 0.02^{a}	1.39 ± 0.01^{a}
Water-soluble carbon (%)	TV1	0.06 ± 0.001^{a}	0.06 ± 0.003^{a}	0.07 ± 0.001^{a}	0.08 ± 0.002^{a}	0.09 ± 0.002^{a}
	TV23	0.07 ± 0.003^{a}	0.08 ± 0.002^{a}	0.08 ± 0.001^{a}	0.09 ± 0.003^{a}	0.10 ± 0.001^{a}
CEC (cmol kg^{-1})	TV1	8.36 ± 0.21^{a}	8.39 ± 0.22^{a}	8.41 ± 0.23^{a}	8.43 ± 0.32^{a}	8.44 ± 0.21^{a}
	TV23	8.38 ± 0.32^{a}	8.40 ± 0.33^{a}	8.42 ± 0.19^{a}	8.43 ± 0.22^{a}	8.47 ± 0.46^{a}
Total N (%)	TV1	0.18 ± 0.001^{a}	0.22 ± 0.001^{a,b}	0.23 ± 0.002^{a,b}	0.25 ± 0.001^{a,b}	0.28 ± 0.003^{b}
	TV23	0.19 ± 0.001^{a}	0.20 ± 0.002^{a}	0.23 ± 0.041^{a,b}	0.24 ± 0.023^{a,b}	0.29 ± 0.029^{b}
Total P as P_2O_5	TV1	9.37 ± 0.12^{a}	9.84 ± 0.39^{a}	9.91 ± 0.42^{a}	9.92 ± 0.23^{a}	9.94 ± 0.49^{a}
	TV23	9.32 ± 0.22^{a}	9.35 ± 0.34^{a}	9.38 ± 0.34^{a}	9.49 ± 0.52^{a}	9.52 ± 0.29^{a}
Total K as K_2O	TV1	94.34 ± 2.39^{a}	95.22 ± 4.29^{a}	95.39 ± 4.98^{a}	95.46 ± 5.69^{a}	95.49 ± 1.29^{a}
	TV23	94.42 ± 3.02^{a}	95.43 ± 3.21^{a}	95.49 ± 4.09^{a}	95.53 ± 2.39^{a}	95.56 ± 3.36^{a}
Total Ni	TV1	2.21 ± 0.13^{a}	2.34 ± 0.14^{a}	2.93 ± 0.14^{a,b}	2.89 ± 0.13^{a,b}	3.12 ± 0.16^{b}
	TV23	2.52 ± 0.14^{a}	2.89 ± 0.15^{a}	3.36 ± 0.17^{b}	3.91 ± 0.17^{b}	4.49 ± 0.11^{c}
DTPA extractable Ni	TV1	0.87 ± 0.04^{a}	0.92 ± 0.04^{a,b}	0.94 ± 0.04^{a,b}	0.96 ± 0.03^{a,b}	1.01 ± 0.06^{b}
	TV23	0.91 ± 0.05^{a}	0.93 ± 0.04^{a,b}	0.94 ± 0.03^{a,b}	0.98 ± 0.04^{a,b}	1.02 ± 0.05^{b}
Different fraction of Ni*						
F1	TV1	0.02 ± 0.001^{a}	0.03 ± 0.001^{a}	0.03 ± 0.001^{a}	0.01 ± 0.001^{a}	0.04 ± 0.002^{a}
	TV23	0.14 ± 0.004^{a}	0.13 ± 0.004^{a}	0.16 ± 0.005^{a,b}	0.18 ± 0.002^{a,b}	0.21 ± 0.009^{b}
F2	TV1	0.12 ± 0.002^{a}	0.22 ± 0.002^{b}	0.21 ± 0.003^{b}	0.31 ± 0.003^{c}	0.32 ± 0.003^{c}
	TV23	0.09 ± 0.001^{a}	0.10 ± 0.001^{a}	0.16 ± 0.002^{b}	0.14 ± 0.002^{a,b}	0.17 ± 0.002^{b}
F3	TV1	0.73 ± 0.02^{a}	0.67 ± 0.02^{a,b}	0.70 ± 0.03^{a,b}	0.64 ± 0.02^{b}	0.65 ± 0.02^{b}
	TV23	0.68 ± 0.02^{a}	0.70 ± 0.03^{a}	0.62 ± 0.03^{a}	0.66 ± 0.03^{a}	0.64 ± 0.02^{a}
F4	TV1	0.22 ± 0.003^{a}	0.21 ± 0.002^{a}	0.11 ± 0.002^{b}	0.09 ± 0.001^{b}	0.07 ± 0.001^{b}
	TV23	0.23 ± 0.002^{a}	0.33 ± 0.002^{b}	0.42 ± 0.003^{b}	0.78 ± 0.06^{c}	0.99 ± 0.06^{d}
F5	TV1	0.91 ± 0.05^{a}	0.98 ± 0.04^{a}	0.34 ± 0.006^{b}	0.46 ± 0.004^{b}	0.72 ± 0.03^{c}
	TV23	1.02 ± 0.05^{a}	1.00 ± 0.05^{a}	1.09 ± 0.05^{a}	1.19 ± 0.05^{a}	1.78 ± 0.06^{b}
F6	TV1	0.21 ± 0.002^{a}	0.23 ± 0.002^{a}	1.54 ± 0.09^{b}	1.38 ± 0.06^{b}	1.32 ± 0.05^{b}
	TV23	0.36 ± 0.004^{a}	0.63 ± 0.04^{b}	0.91 ± 0.05^{c}	0.96 ± 0.05^{c}	0.70 ± 0.05^{b}

† We did not find any significant changes in control treatment with respect to initial experimental soil; therefore, no data for T0 (soil without MSWC) are given here

‡ T0: no MSWC; T1: 2 t ha^{-1} MSWC; T2: 4 t ha^{-1} MSWC; T3: 6 t ha^{-1} MSWC; T4: 8 t ha^{-1} MSWC; and T5: 10 t ha^{-1} MSWC

* F1: water-soluble, F2: exchangeable, F3: bound to carbonates, F4: bound to Fe and Mn, F5: organically bound and F6: residual fractions

Same symbol within row indicates there is no significant difference between treatments, and different letters indicate the pair of treatments are significantly different at 5 % level of significance

amendments, particularly in acidic soil. The increase in the soil pH might be due to the mineralization of high molecular carbon compounds and subsequent production of OH^{-} ions by ligand exchange as well as release of basic cations. Shiralipour et al. (1992) reported that application of MSWC increased the pH of acid soil, and this was due to neutral or slightly alkaline pH of MSWC as well as high buffering capacity of MSWC. It was also reported that on application of compost to acid soil having pH below 5.5, the pH was elevated in addition to reduction or elimination of Al or Mn toxicity (García-Gil et al. 2004). Mohammad and Athamneh (2004) reported decrease in soil pH from alkaline to neutral after addition of MSWC amendment due to decomposition and mineralization of the organic matter, which increased the CO_2 levels and decreased the pH. Incorporation of MSWC in soil slightly increased soil EC, and due to use of relatively lower amounts of MSWC in soil, the changes in EC values in amended soils were insignificant with small increment of MSWC application in the study. Changes in EC in amended soil could be due to high EC levels of MSWC (3.36 ± 0.04 dSm^{-1}), which was attributed to extensive decomposition of organic materials that led to high salt concentrations (Shiralipour et al. 1992). Analysis of one-way ANOVA with DMRT revealed significant change in soil organic carbon content with the increasing load of MSWC irrespective of tea clone used. The maximum increase occurred with the treatment of 10 t MSWC ha^{-1}. Although, high dose of MSWC application (e.g., 617 mt ha^{-1}) had been documented by Shiralipour et al. (1992), comparatively lower amounts of MSWC were used in the present experiment by taking into consideration of its possible environmental hazards (Karak et al. 2014b).

Use of MSWC in agriculture not only helps disposing these materials cost-free, but also reduces its negative impact on the environment in addition to improving soil properties. The addition of MSWC improves soil organic matter status, which in turn stabilizes as well as improves the soil structure and aeration. Improvement of soil physical and chemical properties on application of MSWC had been reported by other researchers also (Karak et al. 2014a, 2015c).

Soil Ni contents after application of MSWC is presented in Table 2, which shows the Ni concentrations (pseudo total, DTPA extractable and different fractions of Ni) in the control and MSWC-amended soil after 1 month of treatment imposition for two selected tea clones. The pseudo total concentration of Ni showed statistically significant differences ($p < 0.05$) compared to the control soil; however, no differences on Ni distribution pattern were observed for different clones with particular treatments.

Fractionation and risk assessment of nickel in soil

We reported data pertaining to Ni but not Cr in the study although Cr contents in soil and MSWC were almost comparable to those of Ni. Cr is not reported in this study as a profile of Cr present in MSWC, and its effect in soil had already been reported from an earlier study (Karak et al. 2014b). Sequential extraction results of Ni in soil influenced by treatments and different clones of tea plants are presented in Fig. 1a. Nickel for each chemical fraction is presented in terms of the percent (%) extracted from the total metal concentration. The present study clearly indicates that amendment of MSWC in soil increased the pseudo total concentrations of Ni as compared to the control soil as well as increased the concentration of Ni in all the fractions. For TV1 clone, Ni distribution in control soil is as follows: residual fractions > bound to Fe and Mn > organically bound > exchangeable > bound to carbonates > water-soluble. However, for TV23 clone it was found in the following order: residual fractions > organically bound > bound to Fe and Mn > exchangeable > water-soluble > bound to carbonates. The dragged order of Ni in different fractions for TV1 clone in decreasing order is: organically bound > bound to Fe and Mn $\gg$ residual fractions > bound to carbonates > exchangeable $\approx$ water-soluble and for TV23 clone it was: organically bound $\approx$ residual fractions > bound to Fe and Mn > exchangeable > water-soluble $\approx$ bound to carbonates, indicating the effect of the clone. In all the cases, Ni is dominantly bound in Fe and Mn oxide (F4), organic (F5) and residual fractions (F6). In F4 fraction, Ni is bound up to 50.6 % of the pseudo total Ni. However, Ni in F5 and F6 was bound to 56.7 and 59.2 % of pseudo total Ni, respectively. High amount of Ni in F5 could be due to the presence of organic matter mineralization in compost in the long term as well as high organic carbon content in tea growing soil (Karak et al. 2011; Paradelo et al. 2011). On the other hand, Achiba et al. (2009) explained that formation of insoluble organometallic complexes of Ni encourages the high amount of Ni in organic fraction. Comparatively, higher percentage of Ni in Fe–Mn oxide bound fraction could be due to the presence of higher amount of Fe and Mn in acidic tea growing soils (Karak et al. 2011). This finding is in agreement with Rajapaksha et al. (2012) where the authors reported that Ni is mainly bound in Fe–Mn oxide fraction in lateritic (Inceptisol) soil from the Ussangoda, Ambalantota in Southern Sri Lanka. In this study, the available Ni in amended soils ranged from 11 to 21 % of the pseudo total Ni; similar percentage (15 %) was observed by Carbonell et al. (2011) in MSWC-amended soil. Clonal variation significantly varied the labile forms (F1, F2 and F3 fractions) irrespective of the treatments imposed. For example, when 2 t ha^{-1} MSWC

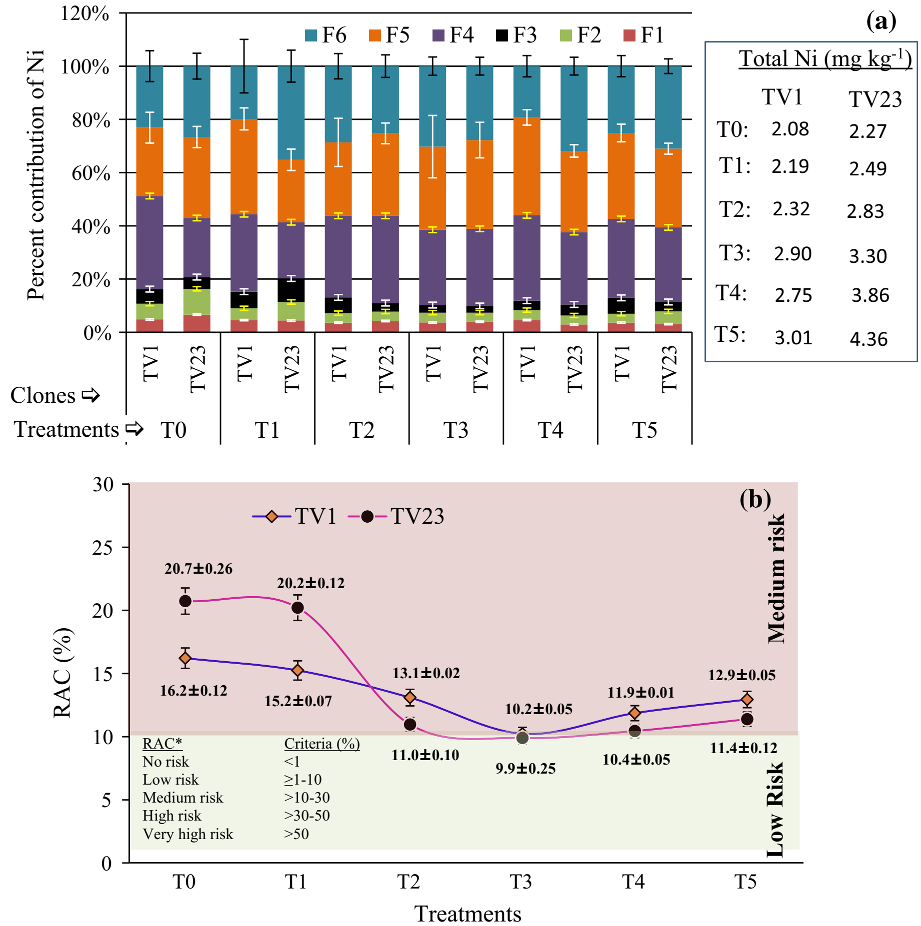

Fig. 1 **a** Distribution pattern of Ni in different fractions with the variation of different doses of MSWC amendments in soils grown with TV1 and TV23 clones (F1: water-soluble, F2: exchangeable, F3: bound to carbonates, F4: bound to Fe and Mn, F5: organically bound and F6: residual fractions); **b** classification of the investigated soils after MSWC amendments

was applied to TV1 and TV23 clone, 16.1 % of the labile fraction of Ni was found in TV1; however, it was 24.2 % for TV23 clone. Similarly, labile fraction of Ni for TV1 clone was found as 19.4 %, and for TV23 clone, it was found to be 18.8 % when 10 t ha^{-1} MSWC was applied to TV1 and TV23 clones. Therefore, it must be considered to speculate trace elements in tea growing soil amended with MSWC varied tea clones on individual basis in order to predict their behavior in MSWC-amended soil (Karak et al. 2015a).

Figure 1b shows the RAC for the amended soil at different application rates. All treatments, including the control treatment (without MSWC), offered a medium environmental risk. However, it has been observed that the Ni concentration in soil increased when the MSWC application doses were raised to result exacerbated Ni accumulation in soil (please see Table 2). Moreover, the possibility of metal leaching from soil to groundwater bodies, as well as runoff to reach superficial waters, must be taken into account. Furthermore, there are conflicting explanations on the phytoavailability of heavy metals in MSWC-amended soil. For example, the presence of high chemically active sites in MSWC makes Ni less available in soil in spite of Ni being a constituent of MSWC (Zheljazkov and Warman 2004). However, some studies postulated that organic fraction of MSWC hindered Ni phytoavailability as well as risk of Ni contamination (McLaren and Clucas 2001). Therefore, such uncertainty leads to some kinds of dilemma over the consequences of MSWC application, which involves the need to estimate the risk assessment of Ni when MSWC is applied in agricultural soils. Figure 1b shows the results of the risk assessment code (RAC) of Ni, which is supposed to be a good indicator on the metal pollution risk in agricultural soil amended with MSWC. Despite the presence of Ni contents in MSWC, the addition of MSWC reduced the RAC (from medium risk to low risk) with the proportional increased dose of MSWC applied from 2 to 6 t ha^{-1}. However, the slight increase in RAC was observed in Ni while the increment of MSWC application rate was from 8 to 10 t ha^{-1}. Paradelo et al. (2011) reported that high organic carbon content of MSWC could have affinity toward some toxic elements, and therefore, MSWC acts as a sink for contaminants. Karak et al. (2010) also reported that MSWC has a great affinity for retaining trace elements in non-available forms that potentially reduce their overall

Table 3 DTPA-extractable Ni in soil, dry matter yield, Ni concentrations in tea and tolerance index (T_i) as influenced by MSWC rates (values represent the mean of three replications ± SEM)

Treatments*	DTPA-Ni ($mg\ kg^{-1}$) in soil	Dry matter yield (g per plant)				Plant Ni ($mg\ kg^{-1}$)				T_i
		Main root	Feeder root	Stem	Leaves	Main root	Feeder root	Stem	Leaves	
Clone TV1 (quality clone)										
T0	$0.37 \pm 0.01^{\dagger a}$	5.31 ± 1.09^{a}	0.37 ± 0.29^{b}	4.07 ± 0.58^{c}	5.93 ± 0.78^{d}	9.18 ± 0.18^{e}	8.11 ± 0.11^{e}	8.02 ± 0.06^{e}	5.65 ± 0.35^{e}	1.00 ± 0.0
T1	0.39 ± 0.02^{a}	8.66 ± 6.81^{a}	0.36 ± 0.41^{b}	$7.60 \pm 1.81^{b,c}$	$9.22 \pm 0.51^{c,d}$	9.69 ± 0.41^{e}	8.40 ± 0.12^{e}	9.17 ± 0.15^{d}	6.15 ± 0.09^{d}	1.65 ± 0.01^{a}
T2	0.32 ± 0.03^{a}	8.94 ± 1.58^{a}	0.68 ± 0.51^{b}	$9.04 \pm 0.94^{b,c}$	$12.21 \pm 0.90^{b,c}$	10.48 ± 0.46^{d}	9.15 ± 0.14^{d}	11.17 ± 0.10^{c}	6.86 ± 0.21^{c}	1.97 ± 0.01^{a}
T3	0.33 ± 0.01^{a}	10.15 ± 1.47^{a}	$1.51 \pm 0.73^{a,b}$	12.86 ± 1.05^{b}	$13.39 \pm 0.12^{a,b}$	11.19 ± 0.44^{c}	9.97 ± 0.05^{c}	11.64 ± 0.60^{c}	8.00 ± 0.11^{b}	$2.42 \pm 0.02^{a,b}$
T4	0.37 ± 0.02^{a}	12.58 ± 2.65^{a}	2.03 ± 0.17^{a}	$12.12 \pm 1.17^{b,c}$	$12.63 \pm 0.41^{a,b}$	12.04 ± 0.17^{b}	10.94 ± 0.07^{b}	12.89 ± 0.27^{b}	8.26 ± 0.07^{b}	$2.51 \pm 0.01^{a,b}$
T5	0.40 ± 0.04^{a}	11.31 ± 2.01^{a}	2.60 ± 0.48^{a}	27.66 ± 0.86^{a}	15.72 ± 1.72^{a}	12.72 ± 0.32^{a}	11.55 ± 0.39^{a}	15.05 ± 0.20^{a}	8.83 ± 0.18^{a}	3.65 ± 0.03^{b}
Clone TV23 (yield clone)										
T0	0.49 ± 0.06^{a}	8.04 ± 1.49^{b}	1.66 ± 0.70^{b}	16.24 ± 1.31^{b}	5.84 ± 1.03^{b}	13.89 ± 0.11^{d}	10.14 ± 0.14^{e}	8.26 ± 0.09^{f}	7.25 ± 0.15^{d}	–
T1	0.52 ± 0.06^{a}	$14.83 \pm 1.17^{a,b}$	$4.65 \pm 0.15^{a,b}$	$26.21 \pm 2.22^{a,b}$	$17.24 \pm 2.79^{a,b}$	14.39 ± 0.34^{d}	12.69 ± 0.30^{d}	9.25 ± 0.12^{e}	7.83 ± 0.13^{c}	4.01 ± 0.03^{a}
T2	0.34 ± 0.02^{b}	$15.32 \pm 1.46^{a,b}$	$2.81 \pm 0.14^{a,b}$	18.97 ± 2.36^{b}	$11.66 \pm 0.26^{a,b}$	15.33 ± 0.09^{c}	13.53 ± 0.31^{c}	10.43 ± 0.14^{d}	8.38 ± 0.17^{c}	3.11 ± 0.02^{a}
T3	0.36 ± 0.02^{b}	11.65 ± 1.99^{b}	$4.32 \pm 0.21^{a,b}$	$27.33 \pm 9.01^{a,b}$	$15.51 \pm 1.04^{a,b}$	15.83 ± 0.13^{c}	14.52 ± 0.42^{b}	11.73 ± 0.39^{c}	9.06 ± 0.21^{b}	3.75 ± 0.04^{a}
T4	$0.43 \pm 0.02^{a,b}$	$20.13 \pm 1.88^{a,b}$	$3.08 \pm 0.66^{a,b}$	$30.91 \pm 6.97^{a,b}$	$13.53 \pm 2.86^{a,b}$	16.80 ± 0.10^{b}	14.96 ± 0.08^{b}	12.64 ± 0.36^{b}	9.20 ± 0.12^{b}	4.31 ± 0.01^{a}
T5	0.53 ± 0.04^{a}	26.77 ± 0.54^{a}	5.85 ± 0.57^{a}	39.58 ± 3.73^{a}	23.56 ± 5.06^{a}	17.60 ± 0.78^{a}	16.49 ± 0.26^{a}	14.29 ± 0.21^{a}	10.76 ± 0.66^{a}	6.11 ± 0.03^{b}

* T0: no MSWC; T1: 2 $t\ ha^{-1}$ MSWC; T2: 4 $t\ ha^{-1}$ MSWC; T3: 6 $t\ ha^{-1}$ MSWC; T4: 8 $t\ ha^{-1}$ MSWC; and T5: 10 $t\ ha^{-1}$ MSWC

† Same symbol within column indicates no significant difference between treatments, and different letters indicate significant difference between a pair of treatments at 5 % significance level

bioavailability and toxicity. The present study have revealed that increasing MSWC application rates decrease the labile fraction of Ni in soil (please see Fig. 1a), which further have justified the decreasing trend of RAC with respect to the increased doses of MSWC applied.

DTPA extractable Ni in soil, biomass yield, Ni accumulation and tolerance index

Investigation showed significant increase ($p < 0.01$) of pseudo total Ni and DTPA extractable Ni in soil receiving increasing doses of MSWC (Table 3). For TV1 and TV23 clones, the relationship between pseudo total Ni and DTPA extractable Ni were $R^2 = 0.94$ and $R^2 = 0.97$, respectively.

In general, both the clones showed increments in root, stem and leaves biomass at all MSWC application rates (Table 3), and therefore, MSWC amendment did not thwart the enhancement of tea yield. The highest total dry biomass for TV1 was T5 (57.29 ± 3.26 g plant^{-1}) followed by T4 (39.36 ± 1.29 g plant^{-1}) $\approx$ T3 (37.91 ± 1.18 g plant^{-1}), T2 (30.87 ± 1.03 g plant^{-1}) $\approx$ T1 (25.84 ± 0.48 g plant^{-1}) and T0 (15.68 ± 0.28 g plant^{-1}). For TV23 clone, it was T5 (95.76 ± 5.62 g plant^{-1}) > T4 (67.65 ± 2.39 g plant^{-1}) > T2 (62.93 ± 2.29 g plant^{-1}) > T4 (58.81 ± 4.02 g plant^{-1}) > T3 (48.76 ± 3.92 g plant^{-1}) > T0 (31.78 ± 2.29 g plant^{-1}). High biomass production under treatment T5 could be ascribed as the greater nutrient added through high doses of MSWC.

The distribution pattern of Ni in different parts of tea plant considerably varied with treatment and clonal variation (Table 3). Within the plant part, Ni concentration occurred in the order: main root > feeder root > - stem > leaves. The distribution patterns of Ni in plants indicate that transpiration has a large influence in the long run transport within plants. Addition of 6 t ha^{-1} of MSWC to soil increased tissue Ni both in TV1 and in TV23 clones nearly about 1.5-fold with respect to unamended soil. However, further addition of 8 and 10 t ha^{-1} of MSWC application results slight increase in Ni in plant tissue. Both the clones contained similar amounts of Ni; however, because of the greater biomass production, the overall Ni accumulation in TV23 was much greater that TV1 clone. A similar trend was observed for some of the other elements (Cu, Zn and Pb), while source-separated MSWC was applied in soil (20, 40, and 60 % MSWC to soil by volume) to two acidophilic crops like Swiss Chard and Basil (Zheljazkov and Warman 2004). Although the leaves of tea plants represented about one-third of the total plant weight, about 42 % of the total Ni is accumulated in leaves irrespective of treatment imposed. However, Ni was evenly distributed in the other plant parts. Furthermore, we also emphasized on water-soluble Ni in leaves as water infusion from tea leaves is the major source of Ni for human when tea infusion is consumed by human beings (Karak and Bhagat 2010). Only 8.42–12.46 % of total Ni in tea leaves was extracted by water (data not shown), which is much lower acceptable daily intake of Ni (<1 mg day^{-1}) as toxic elements in daily dietary and safety standards (Karak and Bhagat 2010). Therefore, application of MSWC in tea plantation could be an alternative source of organic fertilizers.

In the present study, there were significant differences in tolerance index (T_i) values between two clones. T_i values were within the range from 1.65 to 3.65 for TV1 clone and from 3.11 to 6.11 for TV23 clone among the treatments. In general, TV1 clone was more sensitive to Ni than TV23. A good relationship ($p < 0.05$) between DTPA extractable Ni in soil and total Ni concentration in plant suggested that tea plant has shown Ni tolerance. Tolerance is therefore conferred by the possession of specific physiological mechanisms, which effectively enable it to function normally even in the presence of high concentrations of potential toxic elements. These results are also in line with the work reported by Karak et al. (2010) where the degree of tolerance is mainly governed by the specific metal concentration in contaminated areas.

DMRT results reported in Table 3 showed that in TV1, with respect to DTPA-Ni in soil and dry matter yield in main root, no treatment pairs are significantly different from each other. With respect to dry matter yield in feeder root, T0, T1 and T2 form a homogenous group and T4 and T5 form separate group, whereas T3 can fall in either of the group. With respect to dry matter yield in stem, T5 is significantly different from all other treatments. In leave dry matter, T0 is different from all other treatments except T1. As far as the plant Ni content is concerned, in both main and feeder root, all the pair of treatments are different except the pair T0 and T1; in stem, the treatments T0, T1, T4 and T5 differ pair wise; in T_i, treatment T5 is different from T1 and T2. Similar type of conclusion can be drawn in TV23.

Bioaccumulation factor (BAF) and translocation factor (TF) of Ni in tea plants

Bioaccumulation and translocation factors are the parameters usually used to quantify plant uptake of the metal of interest (Carbonell et al. 2011; Karak et al. 2014b, 2015a). The BAFs in main root, feeder root, stem and leaves after 2 years of growing period are tabulated in Table 4. BAFs data presented in non-italic, italic bold and italic numbers considering pseudo total Ni content in soil, DTPA extractable Ni content in soil and bioavailable fraction (F1 + F2 + F3 fraction) in soil, respectively. In all the cases, BAFs were greater than unity. The BAFs for pseudo

Table 4 Bioaccumulation factor (BAF) and transfer factor (TF) in tea as influenced by MSWC amendments (values represent the mean of three replications ± SEM)

Treatments*	BAF**				TF		
	Main root	Feeder root	Stem	Leaves	C_{stem}/C_{root}	C_{leaves}/C_{stem}	C_{leaves}/C_{root}
Clone TV1 (quality clone)							
T0	4.42 ± 0.32	3.91 ± 0.02	3.86 ± 0.02	2.72 ± 0.03	0.464 ± 0.011$^{\dagger d}$	0.704 ± 0.001^{d}	0.327 ± 0.020^{d}
	24.80 ± 1.09	***21.93*** ± 1.02	***21.68*** ± 1.29	***15.28*** ± 1.23			
	27.26 ± 1.11	*24.10* ± 1.00	*23.83* ± 2.20	*16.79* ± 1.12			
T1	4.43 ± 0.02	3.84 ± 0.02	4.19 ± 0.03	2.81 ± 0.04	0.507 ± 0.028^{c}	0.671 ± 0.004^{c,d}	0.340 ± 0.005^{c,d}
	24.85 ± 1.21	***21.53*** ± 1.04	***23.51*** ± 1.35	***15.78*** ± 1.00			
	29.08 ± 1.34	*25.19* ± 1.23	*27.51* ± 2.46	*18.46* ± 1.11			
T2	4.53 ± 0.05	3.95 ± 0.04	4.82 ± 0.18	2.96 ± 0.02	0.569 ± 0.017^{b}	0.614 ± 0.013^{c}	0.350 ± 0.011^{b,c}
	32.76 ± 2.34	***28.60*** ± 2.03	***34.90*** ± 3.09	***21.45*** ± 1.01			
	34.56 ± 2.39	*30.18* ± 2.39	*36.81* ± 0.99	*22.63* ± 2.09			
T3	3.86 ± 0.05	3.44 ± 0.07	4.01 ± 0.04	2.76 ± 0.01	0.550 ± 0.021^{b}	0.687 ± 0.018^{c}	0.378 ± 0.005^{a}
	33.91 ± 3.09	***30.22*** ± 2.09	***35.27*** ± 3.41	***24.23*** ± 2.03			
	37.72 ± 3.13	*33.62* ± 2.33	*39.24* ± 4.09	*26.96* ± 1.23			
T4	4.37 ± 0.24	3.97 ± 0.01	4.68 ± 0.03	3.00 ± 0.01	0.561 ± 0.014^{b}	0.641 ± 0.024^{c}	0.359 ± 0.003^{a,b}
	32.55 ± 3.11	***29.58*** ± 1.94	***34.83*** ± 2.46	***22.33*** ± 1.23			
	36.87 ± 2.96	*33.50* ± 1.48	*39.45* ± 3.09	*25.30* ± 1.00			
T5	4.22 ± 0.03	3.83 ± 0.04	5.00 ± 0.09	2.93 ± 0.01	0.620 ± 0.009^{a}	0.587 ± 0.031^{c}	0.364 ± 0.007^{a,b}
	31.81 ± 1.29	***28.88*** ± 1.29	***37.63*** ± 3.05	***22.08*** ± 1.23			
	32.62 ± 3.01	*29.62* ± 2.09	*38.60* ± 2.33	*22.64* ± 1.28			
Clone TV23 (yield clone)							
T0	6.13 ± 0.28	4.47 ± 0.02	4.05 ± 0.09	3.20 ± 0.01	0.344 ± 0.021^{d}	0.878 ± 0.344^{b}	0.302 ± 0.006^{a,b}
	28.34 ± 1.02	***20.69*** ± 1.02	***18.71*** ± 0.84	***14.80*** ± 1.02			
	29.55 ± 1.11	*21.57* ± 1.34	*17.58* ± 0.45	*15.43* ± 1.01			
T1	5.78 ± 0.05	5.10 ± 0.29	3.71 ± 0.01	3.15 ± 0.02	0.342 ± 0.010^{d}	0.846 ± 0.041^{b}	0.289 ± 0.005^{b}
	27.68 ± 1.29	***24.40*** ± 1.28	***17.78*** ± 0.99	***15.06*** ± 1.02			
	28.60 ± 1.22	*25.21* ± 1.22	*18.37* ± 1.09	*15.56* ± 1.09			
T2	5.42 ± 0.28	4.79 ± 0.29	3.69 ± 0.09	2.96 ± 0.04	0.361 ± 0.042^{c}	0.803 ± 0.016^{b}	0.290 ± 0.006^{b}
	45.08 ± 3.49	***39.79*** ± 2.39	***30.68*** ± 5.09	***24.64*** ± 1.38			
	49.44 ± 4.23	*43.65* ± 3.49	*33.65* ± 4.09	*27.02* ± 1.28			
T3	4.80 ± 0.09	4.40 ± 0.29	3.55 ± 0.08	2.75 ± 0.04	0.386 ± 0.049^{b}	0.772 ± 0.022^{a,b}	0.298 ± 0.007^{b}
	43.98 ± 3.49	***40.33*** ± 3.29	***32.57*** ± 3.09	***25.17*** ± 1.05			
	48.47 ± 4.03	*44.45* ± 3.12	*35.90* ± 4.05	*27.73* ± 1.28			
T4	4.35 ± 0.02	3.88 ± 0.19	3.27 ± 0.02	2.38 ± 0.02	0.398 ± 0.056^{b}	0.728 ± 0.019^{a}	0.290 ± 0.004^{b}
	39.07 ± 1.29	***34.79*** ± 3.02	***29.39*** ± 2.11	***21.40*** ± 1.28			
	41.65 ± 2.09	*37.09* ± 2.09	*31.33* ± 3.46	*22.81* ± 1.26			
T5	4.03 ± 0.05	3.78 ± 0.78	3.28 ± 0.22	2.47 ± 0.03	0.419 ± 0.038^{a}	0.753 ± 0.017^{a}	0.316 ± 0.019^{a}
	33.20 ± 2.22	***31.11*** ± 2.78	***26.97*** ± 2.07	***20.30*** ± 0.04			
	35.43 ± 1.49	*33.19* ± 2.27	*28.78* ± 2.11	*21.66* ± 0.98			

* T0: no MSWC; T1: 2 t ha^{-1} MSWC; T2: 4 t ha^{-1} MSWC; T3: 6 t ha^{-1} MSWC; T4: 8 t ha^{-1} MSWC; and T5: 10 t ha^{-1} MSWC

** BAF - non-italic numbers: BAF as pseudo total Ni content in soil; ***Italic bold*** numbers: BAF as DTPA extractable Ni content in soil; *Italic* numbers: BAF as Ni bioavailable fraction (F1 + F2 + F3 fraction) in soil

† Same symbol within column indicates no significant difference between treatments, and different letters indicate significant difference between a pair of treatments at 5 % significance level

total Ni were <5 in all cases; however, when considering the DTPA extractable and bioavailable fraction (F1 + F2 + F3 fraction) of Ni in soil, the BAFs were in similar order (>15). Carbonell et al. (2011) observed a similar trend for BAFs in a study carried out with maize plants when considering the pseudo total/available fraction of metals; so these authors concluded that available metals in soil provide much more information than pseudo total metal. Higher similar values of BAFs for Ni were obtained for root and stem, suggesting a good translocation from roots to aerial parts. It can be observed that Ni also was distributed along the stem reaching the leaves, as BAF values indicate. Significant differences ($p < 0.05$) between roots and aerial parts were not found. On the contrary, in other works, significant differences have been observed, which may have been the result of the metal-binding capacity of roots because metals strongly bind to the compost matrix and organic matter, thus limiting their solubility and potential bioavailability in soil (Carbonell et al. 2011). Accumulation of Ni in different parts of tea plant could be due to acidophilic nature of the tea plant. Karak et al. (2010) pointed out that Ni accumulation in different parts of tea plant was related to the Ni content in growing soil, which had been further alleviated by acidic soil pH that increased dissolution of heavy metals like Ni where tea plants were grown. A recent study demonstrated that the presence of Ni in tea was due to highly acidic condition of tea growing soil, where Ni was potentially more bioavailable for root uptake (Seenivasan et al. 2008).

A perusal of significant differences in Ni concentration among the different parts of tea plants has been further emphasized by significant higher ratio of C_{stem}/C_{root} (0.46–0.62 for TV1 clone and 0.34–0.42 for TV23 clone). The TFs of C_{stem}/C_{root} were lower than 1 for all treatments. The TF of C_{leaves}/C_{stem} and C_{leaves}/C_{root} was also <1 irrespective of clone used. There was no particular trend of TF with the increasing dose of MSWC applied. The lower TF of C_{leaves}/C_{stem} and C_{leaves}/C_{root} (0.52–0.88) indicated that tea plant followed inclusion strategy. This has also further confirmed that tea roots act as a buffer for translocation of Ni from the root to the parts of tea plant that are above the ground. A similar report has been documented by Bose and Bhattacharyya (2008) where wheat plants were grown in sludge amended soil. As depicted in Table 4; in the clone TV1, on the basis of the ratio C_{stem}/C_{root}, T2, T3, T4 form a single group and remaining each treatment is different from other. With respect to the ratio C_{leaves}/C_{stem}, T2 to T5 forms a homogenous group and T0 and T1 form a different group. Application of 2 t MSWC per hectare of soil can fall in either of the group. On the basis of C_{leaves}/C_{root}, T0 is significantly different from all other treatments except T1. In the similar line, from Table 4, for TV23, different combinations can be explained.

Ni in tea infusion

Table 5 shows the significant differences in the content of Ni in tea infusion were generally observed with different

Table 5 Ni from black tea to infusion as influenced by MSWC amendments (values represent the mean of three replications ± SD, $n = 3$)

Treatments*	TV1	TV23
	Ni in tea infusion (μg L^{-1})	
T0	0.002 ± 0.001	0.01 ± 0.001
T1	0.16 ± 0.001	0.09 ± 0.014
T2	0.39 ± 0.011	0.29 ± 0.027
T3	0.72 ± 0.021	0.46 ± 0.021
T4	0.81 ± 0.030	0.89 ± 0.034
T5	1.21 ± 0.041	1.11 ± 0.028

* T0: no MSWC; T1: 2 t ha^{-1} MSWC; T2: 4 t ha^{-1} MSWC; T3: 6 t ha^{-1} MSWC; T4: 8 t ha^{-1} MSWC; and T5: 10 t ha^{-1} MSWC

Table 6 Pearson's correlation coefficients between biomass and other variables in TV1 and TV23 along with their statistical significance

Parameters	Clone: TV1			
	Biomass			
	Main root	Feeder root	Stem	Leaf
Ni in main root	0.50*	0.827**	0.732**	0.826**
Ni in feeder root	0.420*	0.839**	0.757**	0.802**
Ni in stem	0.402*	0.774**	0.827**	0.862**
Ni in leaf	0.449*	0.813**	0.729**	0.841**
F1	0.249	0.531*	0.334	0.250
F2	−0.124	−0.067	−0.053	−0.387
F3	0.139	0.089	0.412*	0.246
F4	0.619**	0.623**	0.364	0.486
F5	0.404*	0.745**	0.583*	0.720**
F6	0.302	0.105	0.282	0.473*
RAC	−0.398	−0.534*	−0.374	−0.727**
Clone: TV23				
Ni in main root	0.657**	0.436*	0.622**	0.564*
Ni in feeder root	0.584*	0.420*	0.563*	0.530*
Ni in stem	0.575*	0.406*	0.577*	0.472*
Ni in leaf	0.623**	0.448*	0.607**	0.603**
F1	−0.131	−0.111	0.094	−0.264
F2	0.088	0.058	0.101	0.162
F3	−0.005	0.071	−0.013	0.097
F4	0.548*	0.375	0.502*	0.458*
F5	0.372	0.129	0.365	0.219
F6	0.499*	0.398	0.546*	0.497*
RAC	−0.300	−0.153	−0.274	−0.197

* Significant difference at 5 % level and ** significant at 1 % level

treatment of MSWC application. Nickel content in tea infusion produced from TV1 clone was in the range of 0.002 ± 0.001–1.21 ± 0.041 µg L^{-1}, but for TV23 clone it was 0.01 ± 0.001–1.11 ± 0.028 µg L^{-1}. A lowest and highest Ni content in tea infusion was found in TV1 clone in control treatment and TV1 clone in T5 treatment, respectively. Ni in tea infusion was positively correlated with the level of Ni in the tea leaves. This finding also supports the results reported by Seenivasan et al. (2008) when black teas from south India were brewed for 5 min. Till date, there is no defined maximum acceptable concentration of Ni in tea infusion, but present study shows that Ni concentration in per cup tea infusion was below the maximum acceptable concentration of total Ni allowed in beverages (<1 mg day^{-1}) as prescribed by Karak and Bhagat (2010).

Statistical interpretation

Pearson's correlation coefficients were computed for different pairs of variables separately for TV1 as well as TV23, which are presented in Table 6. A perusal of Table 6 indicated that, both in TV1 and in TV23, Ni content in main root, feeder root, stem and leaf was significantly correlated with the biomass. In TV1, F1 was correlated with feeder root biomass; F3 was correlated with stem biomass; F4 was correlated with main root and feeder root biomass; F5 was correlated with all the biomass and F6 was correlated with leaf biomass, whereas RAC was significantly negatively correlated with feeder root and leaf biomass. In the clone TV23, F4 and F6 were significantly correlated with all the biomass except feeder root biomass. In this clone, no significant correlation was found between RAC and biomass.

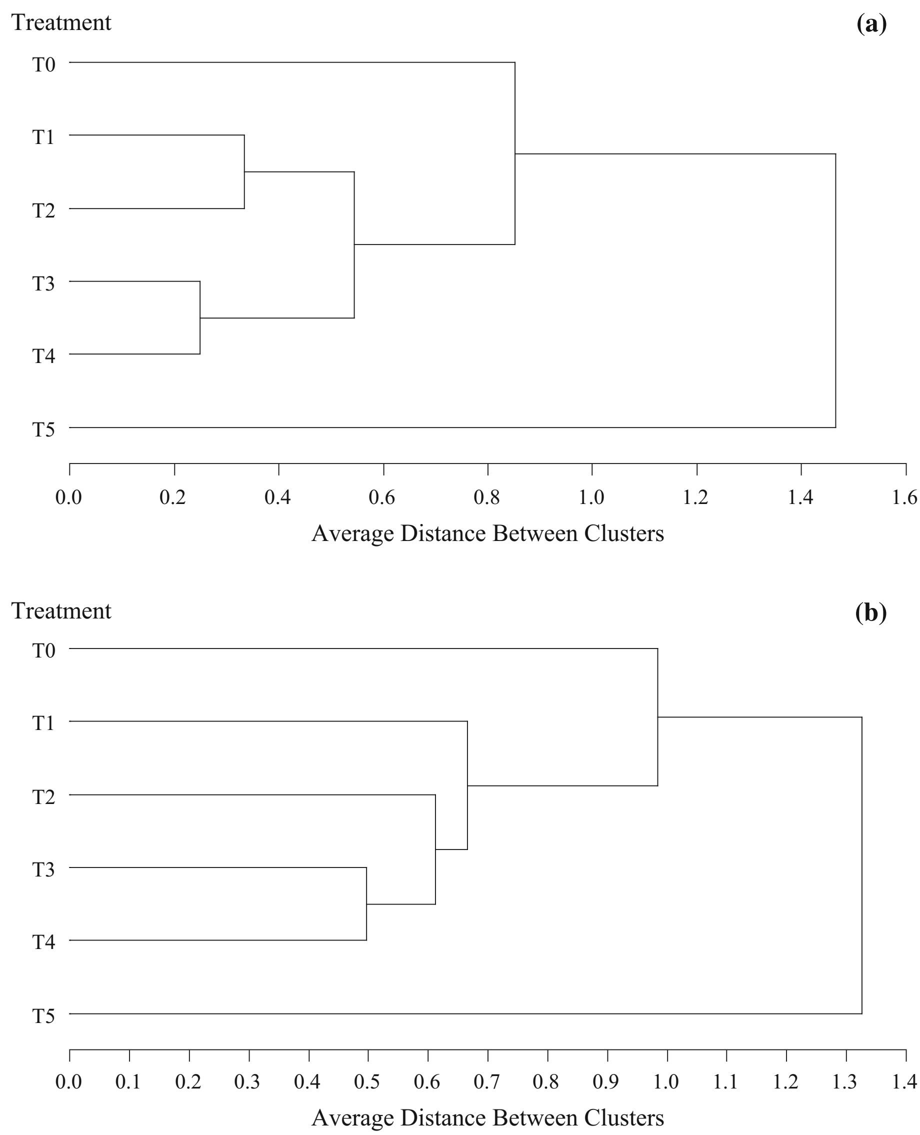

Fig. 2 Dendrogram representing clustering of treatments based on different parameters for **a** TV1, **b** TV23

The dendrograms presented in Fig. 2a, b resulted from hierarchical cluster analysis reveal that, in both TV1 and TV23, three homogenous groups are found: T0 in group 1, treatment T1–T4 form group 2 and T5 fall in group 3. From the present study, it can be concluded that Ni contents in tea infusion grown in MSWC-amended soil did not pose any health problem as it contains quite low amount of Ni. Overall Ni uptake by tea plant and soil risk assessment up to application of 6 t ha^{-1} of MSWC in tea soil were found to be safe. Hence, application of MSWC up to a maximum of 8 t ha^{-1} in tea soil may be recommended on the basis of the present study. However, the present study provides only the experimental results from 2 years of observation with pot experiment. But tea plant being a perennial crop, it deserves long-term experiment for further investigation. Furthermore, the physical and chemical makeup of generated MSW tends to shift with time and source that changes the quality of MSWC. Thus, careful yearly monitoring of quality of MSWC is required. The correlations among biomass in different parts of plant and other variables were computed both in TV1 and in TV23. It is found that RAC is significantly correlated with feeder root biomass and leave biomass in TV1. The pair-wise differences in treatment means with respect to each variable were revealed by DMRT. Using cluster analysis, in both TV1 and TV23, we could also classify the six treatments in three distinct homogenous groups based on all the studied variables.

Conclusion

From the study, it could be concluded that Ni contents in the infusion of tea grown in MSWC-amended soil did not likely lead to health problem as it contained low amount of Ni. Overall Ni uptake by tea plant and soil risk assessment up to application of 6 t ha^{-1} of MSWC in tea soil were found to be safe. Hence, application of MSWC up to a maximum of 6 t ha^{-1} in tea soil could be recommended on the basis of this study. However, the study provided information on two TV clones and that too from 2 years of pot experimentation only. But tea being a perennial crop would deserve long-term pot as well as field experimentations on all the major tea varieties for further investigation. Furthermore, the physical and chemical makeups of generated MSW would tend to shift with time and source, and in turn variation in quality of MSWC. Thus, careful yearly monitoring of quality of MSWC would also be required for. The correlations among biomass of different plant parts and other variables were computed both in TV1 and in TV23. It was found that RAC was significantly correlated with feeder root biomass and leaf biomass in TV1. The pair-wise differences in treatment means with respect to each variable had been revealed by DMRT. Using cluster analysis in both TV1 and TV23, we succeeded in classifying the six treatments in three distinct homogenous groups based on all the studied variables.

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Compliance with ethical standards

Conflict of interest The authors declare no competing financial interest.

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