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Abstract

Municipal solid waste compost (MSWC) is quite often used forsoil amendment inagricultural crops andyet little is known about its effect on tea (*Camellia sinensis* L.) cultivation. This study investigated the effect of MSWC application on cadmium (Cd) concentrations in soil, tea plants and infusions. Different doses of MSWC were added to soil with two Tocklai Vegetative (TV) tea clones (TV1 and TV23)for two years in pot experiments. Several fractions of Cd in amended soils, biomass yield, Cd contents in different parts of tea plants and in tea infusionswere measured. Several indexes related to risk assessmenthad also been calculated.The geo-accumulation index values of Cd in soil amended with MSWC ranged from --1.74 to 3.12 indicating class 0 (practically uncontaminated)to class 4(heavily contaminated) contamination level. Average daily intakes(ADI) of Cdthrough tea infusion produced from tea plant amended with MSWC were $8.9\times10^{--6}$ and $9.2\times10^{--6}$mg kg^{--1}per body weight and day for men and women, respectively, were estimated. Such values weremuch lower than those given in the Joint FAO/WHO Provisional Tolerable Monthly Intake Guideline for Cd. The non-carcinogenic risk values(also called hazard quotient)for Cd, estimated from the ADIvalueswere found to be $1.78\times10^{--2}$ and $1.94\times10^{--2}$mg kg^{-1} per day for men and women,respectively, suggesting no health hazard. The results suggested the possibility of management of MSW through composting and the feasibility of compost application for tea cultivation.

Abbreviations:ADI, average daily intake; BW, body weight; CEC, cation exchange capacity;EC, electrical conductivity;FAAS, flame atomic absorption spectrophotometry;FCOI, Fertilizer Corporation of India;FR, feeder root; HM, heavy metal; HQ, hazard quotient; I_{geo}, geo-accumulation index; L, leave; MR, main root;MSW, municipal solid waste; MSWC, municipal solid waste compost; RAC, risk assessment code; RfD, reference dose; SRM, standard reference materials; ST, stem; TF, translocationfactor; TV, Tocklai vegetative; T_i, tolerance index

Keywords:*Camellia sinensis* L.,Geo-accumulation index,Health risk,Heavy metals, Municipal solid waste

compost

1. Introduction

In agricultural soils,application of municipal solid waste compost (MSWC) becomes a common practice nowadays.Itis progressively gaining popularity due to its ability to improve several soil biological, chemical and physical properties over commonly used organic matter (e.g. cow dung, farmyard manure etc.),which is oftenrare and also expensive[1]. However, MSWC prepared from non-segregated MSW may have adverse effect on application in agricultural soils due to thepossibility of containing several toxic metals including cadmium(Cd) in it[2]. Cd is a highly toxic element in soil as it impedes different chemical processes. Furthermore, Cd can threaten human health when plants grown in soils amended by contaminated MSWC and cadmium-containing food produced from those plants are consumed by human beings[2, 3]. Considerable research has been published in recent decades on MSWC application, primarilyfocused on common agricultural crops (e.g. barley, clover, grape vines, rice, shrubs, spinach, wheat,etc.).Several studies concluded that MSWC could have adverse effect on crops as plants can accumulate several soluble toxic elements from soil [1, 4, 5]. On the other hand, it has been demonstrated that MSWC can notably increase soil organic carbon[6]as well as provide different essential elements for plants and consequently increase crop biomass[5].

Cadmium generally remains in different chemical forms in soils amended with MSWC and which largely determines soil Cd availability for plant uptake and hence total Cd concentration in soils contaminated by anthropogenic sources would not deliver an accurate image of Cd load and pollution, but easily available fraction does[7]. Sequential extraction of Cd fromsoils using reagents with increasing strength, can be a useful tool to predict its mobility and availability which in turn influence its uptake by plants. Furthermore, available fractions of Cd in soils could be used for the calculation of the so-called risk assessment code (RAC), obtainable through sequential extraction[8]. Besides RAC, the degree of Cd accumulation in soils and plants can be estimated through the calculation of several indexes: i) the geo-accumulation index(I_{geo}), comparing the concentration present in the investigated soils with the average background concentration,ii) the translocation factor (TF), and iii) the tolerance index (T_i),expressed as the ratio between biomass production influenced by a treatment and in the absence of treatment.

Tea (*Camellia sinensis* L.) infusion produced from specially produced young shoots of perennial tea plants(most productive up to an age of 30--35 years and still productive up to the age of 70 years), is a non-alcoholic stimulating beverage next to water and consumed by two third of the world's population[10]. Amongst the tea growing countries, India occupies second top most position with respect to tea production and Assam (a prefecture of North-East India) alone shares over 50% of India's total tea production due to its favorable agro-climatic conditions for tea cultivation [11]. Tea infusion is a major source of several essential nutrients to human body; however recent literature has revealed that the presence of trace elements like Cd in tea is due to the tea plants are being normally grown in highly acidic soils where Cd is potentially more bioavailable for root uptake [10--15].Vermicompost and well decomposed cattle manure are frequently used in tea cultivation for maintaining soil health and long sustaining soil fertility status. Application ofMSWC may solve the scarcity of other kinds of compost in tea estates as well as it can help to solve the problem of MSW management[4, 13]. However, criticism is often being faced on application of MSWC on tea growing soils for its heavy metal contents like Cd. However, hardly any scientific finding on phytotoxic effects is available to substantiate such claim of having toxicity of heavy metal on human health due to application of MSWC in tea plantation[13]. Theprevious screening experiments under glasshouse conditions involving two tea clones (Tocklai vegetative 1,TV 1,and Tocklai vegetative 23,TV23)has revealed that tea plant can accumulate significant amounts of Cr and Al following the application of different doses of MSWC [13, 15]. However, there is dearth of information on uptake of Cd by tea plant from MSWC applied soil and its transfer to tea infusion. Therefore, present study aims at evaluating the effect of MSWC application in soils with respect to i) different fractions of Cd in soil from tea cultivated area, ii) role of Cd on tea yield, iii) distribution pattern of Cd within the tea plant,iv) the transfer of Cd from tea leaf to tea infusion, and v)risk analysis of Cd (both on soil and human health). The study can be considered comprehensive since it takes into account all matrices involved in tea production and consumption and considers plenty of analytical parameters. The wide datasethelped to calculate several indexes (i.e. RAC, I_{geo},TFetc.) which were founduseful in summarizingthe status of the matrices under investigation and comparing the data with the results of other studies.

2. Materials and methods

2.1. Preparation of MSWC

During winter season in 2010, non-segregated MSW was collected from the fish market of Jorhat region in Assam. The collected MSW contained 71% organic material; 9% each of paper and plastics; 2% each of metals and textiles, 1% glass/ceramic and 6% other materials. Prior to composting the collected MSW, the non-degradable components were sorted out and chopped mechanically. MSWC was prepared following the protocol described by Karak et al. [13]by composting for 56 days, which was used for the present study.

2.2. Soilsampling

A protocol described by Karaket al. [13] was followed for collecting soil samples in January 2011 for pot experimentation. The collected soil samples were thoroughly mixed together followed by sieving with 2 mm sieve to get representative soil sample for pot experiment. A portion of the sieved soil sample was stored in air tight plastic containers for further analysis.

2.3. Pot experiment and design

36 earthen pots (upper diameter × bottom diameter × height::77 cm ×41 cm×16 cm) were filled with 10 kg collected soil andwere placed in complete randomized design in a greenhouse maintained near environmental condition. Details of the pot experiments will be available atKarak et al.[13]. Altogether six treatments, viz. T0: Control (without MSWC), T1:7.69 g MSWC, T2: 15.38 g MSWC, T3: 23.08 g MSWC, T4: 30.77 g MSWC, and T5: 38.46 g MSWC (corresponding to 0, 2, 4, 6, 8, and 10 t ha^{-1} of MSWC, respectively) were imposed after a month of plantation.

2.4. Soil sampling and pretreatment

Soil samples were collected fromthe depth of 10 cm after twoyears of treatments using a screw auger (referred to as final soil samples). A protocol described by Rubio and Ure [14] was followed for pretreatment and preservation of soil samples prior to the analysis.

2.5. Plant sampling, pretreatment and biomass production

After soil sampling, two yearsold plants were uprooted using flowing tap water. Thereafter, uprooted plants were thoroughly cleaned with tap water followed by distilled water. Different plant parts, viz. feeder roots (FR), main roots (MR), leaves (L) and stems (ST) were separated from tea plants. Plant parts were separately dried at 75°C until constant weightwas achieved [15]. Dried plant samples were weighted separately forbiomass production and expressed as g/plant. Using an agate mortar, dried plant samples were homogenizedfollowing grinding and then sieved with a 40-mesh screen and stored in porcelain airtight containers for future analysis.

2.6. Analytical methods for soil and compost samples

Soil pH was measured using pH meter (Systronics, India, model 239) following the procedure described by McLean [16]. Soil/watersuspensions,1:5,were used for measurement of soil ECwith a conductivity meter (Systronics, India, model 507). The method by Rhoades [17] was applied for CECin experimental soils. A protocol described by Walkley and Black [18] was adopted for estimation of total soil organic C as well as water-soluble C. Total N was determined by the Kjeldahl digestion method. Methodology developed by Peachey et al. [19] was adopted for estimation of plant available P in soils using UV-vis spectrophotometry (Varian Cary 50 Bio spectrophotometer, Australia). Exchangeable K in soil was determined following the procedure described by Hanway and Heidel [20] using a flame photometer (Systronics, India, model 128). The outline described by FCOIwas applied for total K and P in MSWC[21]. $HCl/HNO_3/HF$, 4:2:2,v/v/v,was used to dissolve heavy metal in soil and compost. The procedure in details can be seen in thepreviously reported article [22]. Plant available metals, viz. Cd, Cr, Cu, Ni and Zn were estimated following the outline described by Lindsay and Norvell [23]. A methodology described by Karaket al. [13, 15] was followed for determiningthe germination index (GI) for phytotoxicity assay of prepared MSWC using Indian mustard (*Brassica campestris* L.; cv. PusaJaikisan) and wheat (*Triticum aestivum* L.; cv.PBW3) seeds. Fractionation of soil Cd was sequentiallydone into six different fractions, viz. water-soluble (F1); exchangeable (F2); bound to carbonates (F3); bound to Fe-Mn oxides (F4); organically bound and bound to sulfides (F5); and residual (F6) fractions following standard procedure described elsewhere [12, 13, 15, 24, 25].

2.7. Plant sample analysis for cadmium

For extraction of total Cd, plant samples were digested using conc.HNO_3:conc.HCl::1:3. Extraction procedure and methods of analysis in details are available in Karak et al. [15]. In brief, mixture was heated for 3 h at 85ºC on a hot plate, until the solubilization of the sample was complete and was then diluted to 25 mL with deionized water in a polycarbonate volumetric flask.A blank digestion was carried out in the same way.Cd was determined using flame atomic absorption spectrophotometry (FAAS; Agilent, Australia,240AA).

2.8. Cd contentin tea infusion

Using traditional method, black tea samples were prepared from collected leaf samples and infusions were prepared following the methods described by Seenivasan et al. [26]. In short, in a 250 mL porcelain beaker, 1 g prepared black tea sample was taken and 150 mL double distilled water was added followed by boiling for 5 minunder stirring intermittently. Thereafter, infusion was filtered through Whatmann 1 filter paper and made up to 200 mL in a volumetric flask. All the tea infusions were prepared in triplicate. Cd in the infusion was directly analyzed using FAAS.

2.9. Quality control

Montana Soil (SRM-2710) and domestic sewage sludge (BCR-144) were used as standard reference materials (SRM) to check the accuracy of instrument as well as analytical results by following the same methodology that used for total metal analysis in soils. The measured values of analysed heavy metals in SRM came very close to the certified values.

2.10. Index calculation

2.10.1. RAC

The following equation described by Singh et al. [8] had been adopted for evaluating RAC:

$$\mathrm{RAC}\,(\%) = \left(\sum_{n=1}^{3} F_{\mathrm{n}} \Big/ \sum_{n=1}^{6} F_{\mathrm{n}} \right) \times 100 \quad ...(1)$$

where F_{n} is the concentration of Cd in the *n*th fraction.

2.10.2. I_{geo}

A protocol described by Müller[9] was employed for I_{geo} to understand the loading pattern of Cd in tea growing soils influenced by MSWC. I_{geo} was computed using the following equation:

$$I_{\mathrm{geo}} = \log_2 (C_{\mathrm{n}} / 1.5\, B_{\mathrm{n}}) \quad ...(2)$$

where C_{n} is the total concentration of Cd (mg kg^{-1}) in soil (either in control soil or soil amended with MSWC) after two years growing period of tea plants and B_{n} is the geochemical background value of Cd (mg kg^{-1}) found in soil (mg kg^{-1}) which is estimated to be 0.02 mg kg^{-1} [27]. The constant 1.5 is used due to the natural fluctuations in the content of a given substance in the environment and very small anthropogenic influences [15].

2.10.3. Tolerance Index

The procedure described by Liang et al. [28] had been used for calculating the tolerance index (T_{i}):

$$T_{\mathrm{i}} = \mathrm{DM}_{\mathrm{t}} / \mathrm{DM}_{\mathrm{u}} \quad ...(3)$$

where DM_{t} and DM_{u} are the dry matter yields of MSWC-treated and untreated soils, respectively.

2.10.4. TF of Cd

Translocation factor (TF) is calculated as the ratio of Cd concentration in aerial parts to root parts of plant [25] as:

$$\mathrm{TF} = C_{\mathrm{Cd,AE}} / C_{\mathrm{Cd,R}} \quad ...(4)$$

where C is concentration of Cd (mg kg^{-1}), AE and R stand for aerial parts and roots (e.g. feeder root and main root), respectively.

2.10.5. Risk assessment

The ADI of Cd from tea infusion was assessed following the modified equation of the USEPA [29]:

$\mathrm{ADI} = (C \times \mathrm{DI})/\mathrm{BW}$ ...(5)

where ADI is the average daily intake (mg kg^{-1} bwper day,bwstands for body weight), *C* is the Cd concentration in tea infusion (mg L^{-1}), DI is the average daily consume rate of tea infusion for Indian people (0.600 L day^{-1})when annualper capitaconsumption is 755.78 g/person per year [30], and BW is the average body weight with67.4 and 64.9 kg for Indian men and women, respectively (www.arogyadarpan.com/StandardHeightandWeightforIndianMenandWomen.aspx, accessed 3 March2014). Non-carcinogenicrisk can be estimated numerically using the hazard quotient(HQ) which was calculated by the following equation by Karak et al. [30]:

$\mathrm{HQ} = \mathrm{ADI}/\mathrm{RfD}$ ...(6)

where ADI is the average daily intake (mg kg^{-1} bwper day) and RfD is the reference dose of Cd which is 5×10^{-4} mg kg^{-1} bwper dayas prescribed by the USEPA[29]andIRIS(www.epa.gov/iris,accessed 27 February2015).

2. 11. Statistical analysis

All the statistical analysis of the experimental data set was carried out using SAS software version 9.3 (SAS Institute, USA). Plant biomass variables, viz. MR, FR, ST and L were separately computed for TV 1 and TV 23 to determinePearson's correlation coefficients between the variables. For determining significant differences among treatments, one way analysis of variance, ANOVA, was applied. To calculate the significant differences between pairs of treatment means, Duncan's multiple range test, DMRT, was used [31]. An attempt was also made to model MR, FR, ST and L biomass based on the other computed variables. Stepwise regression analysis was performed to select most significant variables for predicting the respective biomass. Hierarchical clustering algorithm was applied in order to identify the presence of homogenous groups among different treatments based on all the investigated variables [32].

3. Results

3.1. Physico-chemical properties of soil and MSWC

Table 1 shows estimated physical and chemical parameters of thesoil and the prepared MSWC. The soil from tea cultivation showed acidicpH[12]and was rich in carbon. K in the investigated soil was found to be 91.3±8.2 mg kg^{-1}.

The pH of MSWC sample was nearly neutral (7.46±0.12). Electrical conductivity (EC) of MSWC was much higher compared with the experimental soil. The amounts of organic C, N, P and K and C/N ratio are in compliance with the critical levels(organic C: ≥12%; N:≥0.80%; Pas P_2O_5≥0.4%; K as K_2O ≥0.4% and C/N <20) prescribed by FCOI[21].Total organic C (TOC) contents in MSWC was found to be higher than the lowest critical level of 12% as specified by the Indian compost standard[21]whereas water-soluble carbon (WSC)constituted about 3.41 % of TOC. Among the selected HMs, total Zn was most abundant both in soil and in MSWC which was followed by Cu, Ni, Cr and Cd in soil and by Cu, Cr, Ni and Cd in the MSWC. DTPA-extractable metals followed the same trend in both matrices, namely Zn>Cu >Ni>Cr >Cd. Both the total and the extractable metal concentrations are higher in MSWC than in soil. Sequential extraction results show that Cd in soil is mainly present in the residual fraction and secondarily in the organically bound one; as expected the highest percentage of Cd was extracted into the later fraction from MSWC. As Table 1 shows, main properties and total metal contents in MSWC fall within the limits prescribed byFCOI [21]; no reference values for DTPA-extractable elements are reported.

3.2. Characteristics of soils after MSWC amendments

Table 2 shows the physical and chemical soil parameters after two years of pot experimentations using variable MSWC doses. First of all, a comparison between the untreated soil before (Table 1) and after (T0, Table 2) two years of tea growing reveals that the latter has a slightly lower content of nutrients and cadmium. As to the effect of MSWC application, the comparison between T0 and T1--T5 soils shows that pH decreased slightly and insignificantly in amended soils for both clones. Higher EC of applied MSWC resulted in an increasing trend of EC with increasing amount of MSWC dose irrespective of tea clones. As to organic matter, MSWC application caused an increase of TOC in soil relative to control treatment. However, there were no statistically significant differences among the treatments. As expected, in all treatments, NPK increased with increasing dose of MSWC.

Total Cd content increased after all treatments. Cadmiumconcentrations in the control soil were undetectable in all fractions, owing to the low total concentration. In all treatments, Cd concentration was below the detection limit in F1 and increased in the order F2<F3<F5<F4<F6, i.e. it was mainly associated with the least available fractions; in particular, the proportion of Cd was low in carbonate fraction (F3), but higher in the fraction bound to Fe and Mn (F4), and reached its maximum values in the residual fraction (F6). The concentrations in F2--F6 increased with increasing dose of MSWC, following the increase in the total amount present in the soil; on the other hand, the percentage distribution of Cd in F2--F6 as a function of the treatment does not show a definite trend, with the possible exception of slight decrease of F6 for T5, suggesting an overall increase in the other fractions.

3.3. RAC and $I_{geo}$of cadmium in soils

RAC values of Cd in soil, affected byMSWC amendment, together with the evaluation criteria, are presented in Fig. 1. RAC could not be calculated for T0 and T1 because all or most values were below the detection limits. The values reported for T2--T5 may be slightly underestimated, since the contribution of F1 (below the detection limit) was not considered. Anyway it can be hypothesized that the contribution of this fraction would be very low. RAC values were in medium risk level, ranging from 15.4 to 29.4%, for most treatments, with the exception of the two highest doses for TV23.

The I_{geo}values for Cd for the two tea clones with varied dose of MSWC application and their interpretation in terms of classes of contamination are listed in Table 3. The Cd I_{geo} values ranged from --1.74 to 3.12, indicating class 0 to class 4 contamination level. TheI_{geo}value for control soil with TV1 and TV23 clones indicated practically uncontaminated soil. However, increasing doses of MSWC for TV1 clone increased I_{geo} values which ranged between 2.23 and 2.74 denoting moderate contamination. For TV23 clones, I_{geo} values for MSWC-treated soils were between 2.41 and 3.12, which indicated moderate to heavy to extreme contamination.

3.4. Biomass, T_i,Cd concentrations and TF of Cd in plants

Figure 2A and Bshows the biomass yield of different parts of tea plant and the corresponding T_i values. The data indicate a significant increase of biomass production with increasing amount of MSWC in both clones of tea plant without visual toxicity symptoms. Comparing the two clones, significantly higher plant biomass yields were obtained in TV23 clone. The total plant biomass was highest in T5 treatment irrespective of tea clone (T_i= 2.92±0.02 for TV1 and T_i =5.45±0.82 for TV23).

Cadmium concentrations in different parts of *C.sinensis* L. for the two tested clones and different rates of MSWC application are depicted in Fig. 3. Concentrations in leaves were below the detection limit (0.20 mg kg^{-1}), so they were not reported in the figure. The trend of Cd accumulation among the plant parts is MR>FR>ST>>L irrespective of treatments as well as tested clone. TV23 accumulated significantly ($p \leq 0.05$) higher amounts of Cd than TV1.

The TF values of Cd within the tested cultivars ranged from 0.82 to1.07 for TV1 clone and from 0.77 to0.99 for TV 23 clone (Fig. 4A and B). Overall, TF values from MR to FR are higher than those from FR to ST for both clones; TF values from ST to leaves are expected to be still lower. A comparison between TV1 and TV23 shows that the transfer of Cd within TV1 takes place at a higher extent than in TV23.

3.5. Cd in tea infusion and health hazard

Cadmium levels in tea infusions produced from TV1 and TV3 clones grown in soils amended with MSWC were all below the detection limit (0.001 mg L^{-1}).A simple simulation was performed. Assuming that Cd concentration in leaves is equal to the detection limit (0.2 mg kg^{-1}) and the amount of the metal present in 1 g of tea is transferred to 150 mL of water during infusion, the concentration in the infusion would be 0.0013 mg L^{-1}, i.e. it should be detectable. The presence of undetectable amounts of Cd in the drink suggests that a small fraction of the metal is transferred from stem to leaves and/or its extractability into hot water is low.

Average daily intakes (ADI) of Cd through tea infusion produced from tea plant amended with MSWC were estimated under the worst scenario conditions, i.e. hypothesizing that the concentration in the drink is equal to the detection limit: the ADI values thus calculated were 8.9×10^{-6} and 9.2×10^{-6}mg kg^{-1}bw per day for men and women, respectively. Such values are much lower than those given by the Joint FAO/WHO Provisional Tolerable Monthly Intake (PTMI) Guideline: 0.025 mg Cd/kg bw per month, corresponding to 8.33×10^{-4}mg kg^{-1}bwper day[33].

The HQ values for Cd, estimated from the ADI values, were found to be 1.78×10^{-2} and $1.94\times10^{-2}$for men and women,respectively. AnHQ value of >>1 suggests significant health hazard[16].

3.6. Statistical Interpretation

All statistical analyses were performed for tested clone, viz. TV1 and TV23 [31]. Table 4presentsPearson's correlation coefficients between different pairs of variables. It was clearly indicated that, in TV1 and TV23, total Cd content in MR, FR and ST was significantly ($0.05<p$) correlated with MR, L and FR biomass. In TV1, F4 is correlated with MR biomass; all fractions except F5 were significantly ($p> 0.05$) correlated with FR and L biomass. Whereas ST biomass was significantly ($p> 0.05$) correlated with all soil Cd fractions. RAC and I_{geo} were significantly and positively correlated with all biomass values except ST biomass and MR biomass, respectively. TF was significantly correlated with all biomass values except for L and ST biomass. Almost similar results had been found for the TV23 clone but there was no correlation between FR biomass and any other variable.

The dendrograms presented in Fig.5A and B, obtained by hierarchical cluster analysis, reveals the presence of homogenous groups, depending on the amount of compost added to the soil: T0--T1, T2--T4, T5 for TV1 and

T0, T1--T2, T3--T5 (with T5 somewhat differentiated from the other two treatments) for TV23[35, 36]. The results of stepwise regression analysis are reported in Table 5. The most influential variables for predicting stem biomass for TV1 areF2, F5, Cd in stem ST/FR ratio and Ti (R^2=0.894). The leaf biomass can be determined mainly by Cd in feeder root and ST/FR(R^2=0.898). Main root biomass can be predicted based on several parameters, and primarily by F4, F5, total Cd, RAC, Cd in stem and Ti (R^2=0.852). The feeder root biomass is explained mostly by F5, Cd in feeder root and Ti (R^2=0.957).Similarly, the most influential variables for predicting stem biomass for TV23 are RAC, Cd in main root,ST/FRratio, concentration of Cd andTi(R^2=0.965). The leaf biomass and main root biomass can be determined mainly by Ti (R^2=0.791 and R^2=0.879) respectively. The feeder root biomass is explained mostly by ST/FR and Ti (R^2=0.701).

4. Discussion

4.1. Physico-chemical properties of soil and MSWC

The features of the soil used for the experiments are in agreement with literature data on tea garden soils. Total nitrogen in soil supported the data reported by Karak et al.[12]who found that the nitrogen contents in tea growing soils varied between 0.24 and 3.60 g kg^{-1}in 991 soil samples collected from the Dibrugarh and Tinsukia District of Upper Assam, India. In additionthe high organic carbon contents are typical for tea garden soils and are probably due to application of organic matter through retention of tea pruning litter, littering of shade tree leaf and pods, direct application of organic manure, etc.[13]. Ruan et al. recently published notably similar results for K concentration in 3396 soil samples collected from 54 counties of 16 main tea-producing provinces in China during the period 2009--2010 and an average of 81 mg kg^{-1} exchangeable K was found in the soil samples[37]. This similarity might be due to similar application rates of fertilizers, rather than to the characteristics of the underlying soils. As to the relatively low pH, Igwe et al. reported that low pH of soils is due to their porous nature, which is believed to have been inherited from the geology: such a porosity accelerating the net loss of base cations through leaching[38].

The MSWC prepared for the pot experiments fulfills the requirements of the Indian legislation from the point of view of the contents of both the main nutrients, for which lower limits exist, and the total heavy metals, for which upper limits are established[21]. The high value of EC is due to the high amount of soluble cations, as suggested by Karak et al.[39].Among several maturity parameters of prepared compost, water soluble carbon (WSC) is considered as one of the most readily biologically active parameters[3].Karak et al. reported that a low amount of WSC in compost, like the one found in the present work, could be due to continuous mineralization of soluble organic compounds, followed by re-polymerization and condensation pathways that lead to the formation of complex organic substances that have a lower solubility in water and tend to flocculate out of solution[40].

Based on soil analysis prior to MSWC amendment, the used soil can be considered to contain natural concentrations of total Cd, Cr, Cu, Ni and Zn as suggested by Kabata-Pendias and Pendias[41].Cadmium mobility is low in the soil, as demonstrated by the high percentage extracted into the residual fraction (F6).

4.2. Characteristics of soils after MSWC amendments and two years of tea growing

Tea plants grown in the experimental soils assimilated nutrients as well as cadmium, as shown by the decrease in the concentrations found in the control soil (T0) in comparison with those present before the beginning of the experiments. The application of MSWC did not appreciably cause affect soil pH, and no trend as a function of the dose of compost was observed. A decrease in pH had been observed in other studies: for example, Oleszczukfound a decrease of soil pH from 8.3 to 8.0 after the addition of MSWC at the rate of 120 t ha^{-1} because of the decomposition and the mineralization of the organic matter, which decreased the pH of MSWC amended soils due to increase inCO_2[2].On the other hand, the increase in EC with increasing amount of MSWC dose is commonly encountered in the literature [1,2]. The lack of a statistically significant increase in organic matter with increasing compost application, which is surprising, was also found by Karaket al. for tea growing soil in Assam, Indiaand can be attributed to the decomposition and mineralization processes of organic carbon which seem to be in balance during the tea plant growing period [11]. The concentrations of the other main nutrients increased upon addition of MSWC.

Like all metals, Cd is not biodegradable, so its concentrations increased with increasing compost dose, but was still lower than the background Cd value for soils in India (0.20 mg Cd per soil, [22]) except for the treatment T5: mean concentrations of Cd for TV1 and TV23 clones at the treatment T5 are equal to and 30 % higher than background values of Cd in Indian soils, respectively. Oleszczukreported that application of Cd-containing MSWC can build up Cd in soils and that the concurrent increase in available K^+, Ca^{2+} and Mg^{2+} may in turn result in increased Cd desorption from the soil [2]. Li et al. estimated that mean concentrations of Cd in mining areas were greater than the both the background value (0.097 mg kg^{-1}) and the Grade II environmental quality standard (0.3 mg kg^{-1}) for soils in China[42].The partitioning of cadmium in fractions F1--F6 did not change appreciably for treatments T1--T4, whereas a slight decrease in the residual fraction was observed for the highest dose of MSWC. Literature data on this subject are controversial. For instance, Oleszczuk[2]reported conspicuous mobilization of Cd after application of municipal sewage sludge composts in pot experiment with *Lepidiumsativum*due to crop uptake as well as leaching of soluble Cd fraction as solubility of other metals like Cr and Al (which compete with Cd for uptake by plants) is low, as observed also in previous worksreported by Karak et al. [13,15]. In contrast, many researchers found that metal mobility decreased upon compost application. For instance, Mani et al. reported that highly humified fractions lead to stable complexation of Cd and hence reduce Cd availability and mobility in soil[43]. Hargreaves et al. remarked that MSWC supply organic matter resistant to decomposition, such as humic substances, which provide medium-term retention of metals, and inorganic residues such as the phosphates, silicates, Fe, Al, and Mn oxide, which most likely favor long-term metal retention[1]. Karaket al. state that composting usually reduces metal solubility, but does not totally eliminate this process[4, 12, 15]. The different conclusions reached in different studies probably arise from the fact that the effect of compost on metal mobility depends on the characteristics of the soil, e.g. texture, pH, organic matter content, which in turn influence the strength of metal binding to soil components. Finally, it must be pointed out that metal availability is also influenced by compost stability: as compost matures, the humic material tends to increase, which promotes metal binding [1].The fractionation of Cd among F1--F6 found in the presentwork reflects the fact that the soil has a low carbonate content, as demonstrated by its acidic pH; Fe and Mn are the major acid-forming ions in tea-growing soils, which are acidic in nature. Similar results were reported by Karaket al. [22].

4.3. Risk assessment code (RAC) and of Index of geo-accumulation (I_{geo})ofcadmium in soils

RAC of Cd in MSWC amended soil gives a clear indication related to mobility of Cd in soil, i.e. of the fraction weakly bound to the soil main components [3].The results show that RAC increased with increasing MSWC dose, reaching a high risk level for treatments T4 and T5 in TV23. Values for TV23 were always higher than TV1: therefore, RAC of Cd in tea-growing soils amended with MSWCwas influenced by clonal variation; similarly, RAC values for Al and Cr were found to be different in the presence of TV1 and TV23 [13, 15]. Even though $I_{geo}$was originally developed for river bottom sediments, it can also be used for assessment of soil contamination by anthropogenic activities [13]. I_{geo}enables the assessment of environmental contamination of Cd. I_{geo} followed the same trend as RAC valuesof the two clones. These results are different from those obtained by Ozores-Hampton et al. [44], who found that the application of MSWC in an acidic soil through field study had no effect on the level of Cd.

4.4. Biomass and Cd concentrations in plants

The positive effect of MSWC on biomass yield of *C.sinensis* L. plants is not unexpected and is in agreement with literature data on the effect of compost on plants. For instance, Warman et al. concluded that repeated applications of MSWC can increase the biomass yield of low bush blueberry (*Vacciniumangustifolium*Ait.) in three acidic soils of Nova Scotia sites as MSWC amendment provided equivalent amounts of plant essential nutrients as chemical fertilizers do[5]. Furthermore, Hargreaves et al. reported that MSWC application is beneficial for crop production for its positive effect on biological, physical, and chemical soil properties as well as for its content of wide ranges of essential macronutrients and micronutrients[1]. Ozores-Hampton et al. also concluded that MSWC application cannot thwart the biomass production of cultivated plants having no Cd accumulation properties[44]. The higher yield obtained with TV23 is presumably due to the stronger physiological structure of this clone over TV1 clone.

The results indicated that even though Cd accumulation in different parts of tea plants increased with increasing doses of MSWC, no Cd toxicity symptoms were shown by the plant indicating no adverse effect of MSWC application. Furthermore, Cd transport from soil to tea plant could be considered having passive transport as MSWC application ranging from 7.69 to 38.46 g MSWC per pot resulted in varied Cd uptake by a factor of nearly 2. Variation in Cd contents in FR, MR, ST and L of tea plant could be due to different compartmental effects like cell wall, plasma membrane etc.

Variable ranges of Cd concentration in tea leaves are reported in the literature. Chen et al. reported that the concentration of Cd in tea leaves of eight tea (*C.sinensis* L.) cultivars in China ranged from 0.03 to 0.08 mg kg^{--1}[45]. These were in good agreement with the levels obtained in previous studies reported by Karak and Bhagat in their review on tea analysis[10]. However, Yemane et al. analyzed five tea clones grown in Wushwush tea plantation farms, Ethiopia and Cd in the leaf tissues was present at undetectable level[46]. On the other hand, a significant amount of Cd (0.05 to 0.38 mg kg^{--1}) in made tea was detected and quantified by Seenivasan et al. when 100 black tea samples were collected from the major tea growing regions of South India[26]. Karak and Bhagat reported the level of Cd in tea samples marketed in Pakistan to be within the range of below the detectable limit to 0.18 mg kg^{--1}[4].

Tolerance limit f or Cdhas not been made available so farby the Prevention of Food Adulteration Act (PFA) of Indiabut permissible limit of Cd in food grains and seed are setat 0.2 mg $kg^{--1}$by WHO [47]. Tea plant is grown basically for production of the uppermost vegetative part (two leaves and a bud) which determines made tea yield [13]. Furthermore, made tea is used for preparing teainfusion intowhich only a small fraction of Cd is transferred.

TF values in nearly all tested clones are less than unity; similar results have been reported by Lianget al. [28]for Cd accumulation and translocation in spinach (*Spinaciaoleracea* L.)grown in selected soils of China and spiked with different doses of Cd as Cd $(NO_3)_2 \cdot 4H_2O$.

Kloke et al.[48] estimated the phytotoxic concentrations of Cd to be 5 to 10 mg kg^{-1} on dry weight basis in sensitive plant species, while Macnicol and Beckett gave the range of 10 to 20 mg kg^{-1} on dry weight basis as critical Cd levels[49]. Bakerand Walkerproposed that a plant containing more than 1 mg g^{-1}of Cd in its leaves on a dry weight basis is called a hyper-accumulator, irrespective of the metal concentration in the soil[50]. In the present study, it has been observed that tea leaves contain much lower concentration of Cd: actually *C.sinensis* L. is not classified asa hyper-accumulator. Furthermore, it could not be a suitable candidate for phytoextraction of Cd from Cd-contaminated soils as it cannot accumulate >100 mg Cd kg^{-1} in shoots [50].However, Cd extraction capability by tea plant in the field should be considered for its effect on the phytoavailability of this metal, as fractionation of Cd may be changed to less phytoavailable forms over time [26].

4.5. Cd in tea infusion and health hazard

The knowledge of the cadmium contents in tea infusion is necessary as tea is habitually drunk by people in this form, and infusions produced from made tea amended with MSWC could cause exposure to Cd. In this study, we found undetectable levels of Cd in tea infusion, which is positive from the point of view of the quality of the latter and of the safeguard of consumers' health. Low Cd concentrations are usually reported in the literature. According to a critical review by Karak and Bhagat [10], Cd content in tea infusion ranged between trace and 0.79 μg L^{-1}. Karak and Bhagatreported that 0.08 μg Cd L^{-1}was found in commonly used black tea infusions purchased in Norway[10].

In any case, risk of Cd intake through consumption of tea infusion is also related to body weight. ADI values, which take this parameter into account, were much lower than the tolerable value issued by FAO/WHO, suggesting that infusions made with the investigated types of tea leaves do not have adverse impacts on human health, at least from the point of view of Cd content[33].Similarly, the low values of HQ show that no carcinogenic hazard is posed by the levels found in the investigated sample. The lower HQ values of Cd in tea infusion consumption could be due to presence of significantly lower amount of Cd concentration in consumed tea infusion [70--74]. Furthermore inthe present study, cancer risk [$\text{Cancer risk} = 1 - \exp(-\text{ADI} \times \text{SF})$ ...(7)

, where ADI is average daily intake of Cd through consumption of tea infusion and SF is slope factor for Cd] has not been calculated as USEPA although classifies Cd as a probable human carcinogen, but has not provided a SF for Cd and the assessment has not been revised since 1987 [29].

4.6. Statistical interpretation

Pearson's correlation coefficients showed a correlation between Cd total and extractable contents, RAC and $I_{geo}$with plant biomass, confirming that an increasing amount of compost, whilst increasing Cd concentrations in soil and plants, improves plant growth.

The clustering found with hierarchical cluster analysis confirms the different compositions of the treatments and the different behavior of the clones. In particular, T0 is highly differentiated from the other treatments for TV23 because the addition of MSWC caused a more relevant effect on soil and plants for this clone. Sample T5 differs from the other ones both for TV1 and, at a lower extent, for TV23.

5. Conclusions

By compiling all the information from the analysis of soil, plant and tea infusion, it can be concluded that treatment T4, corresponding to an annual application of 8t ha^{-1}MSWC in soilsgives better yield of tea plant, whilst keeping the Cd concentration in tea infusion below the tolerable intake level. Presently MSW is regionally available without any cost and, therefore, its application through composting could be a suitable soil organic amendment for sustainable tea cultivation with the recommended dose for producing tea for human consumption. However, total Cd concentrations in soils and plant parts increased with increasing MSWC dose, even if its mobility in soils was reduced: therefore, care should be given to the possibility of accumulation of heavy metals in soils following repeated MSWC treatments over time. Therefore, a long-term monitoring should be planned to check the compliance of soils and plants to the legislative limits and, if necessary, compost application should be stopped if such limits are approached. The use of compost deriving from segregated municipal solid wastes, which should have a lower content of heavy metals, is anyway preferable. Finally, as the present experiment was restricted to pot trial, further field research should be paramount important for the use of MSWC for tea cultivation in a sustainable way.

Conflict of Interest

The authors have declared no conflict of interest

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Figure captions

Fig 1 Classification of the investigated soils with respect to Cd mobility after two years of MSWC amendments in the presence of two tea plant clones (TV1 and TV23) according to the risk assessment code (RAC) (*: The standard RAC values are adopted from Singh et al. [8])

Fig. 2 Concentrations of total Cd in different parts of tea plants as a function of variable amounts of MSWC (error bars indicate standard deviations of three replicated data)

Fig. 3 Dry biomass (g per plant) of different parts of *C. sinensis* L. plants and tolerance index (T_i) after two years of growth in soils amended with MSWC (mean of three replications,error bars indicate standard deviations of three replicated data) (A) TV 1 and (B)TV 23; T0: Control (without MSWC); T1:7.69 g MSWC; T2: 15.38 g MSWC; T3: 23.08 g MSWC; T4: 30.77 g MSWC; and T5: 38.46 g MSWC per 10 kg soil (corresponding to 0; 2; 4; 6; 8 and 10 t ha^{-1} of MSWC, respectively)

Fig.4 Translocation factors (TF) of Cd from feeder root to main root (FR/MR), stem to feeder root (ST/FR) and leaves to stem (L/ST) in tea plants after two years amendments of MSWC (mean of three replications,error bars indicate standard deviations of three replicated data) (A)TV 1 and (B)TV 23; T0: Control (without MSWC); T1:7.69 g MSWC; T2: 15.38 g MSWC; T3: 23.08 g MSWC; T4: 30.77 g MSWC; and T5: 38.46 g MSWC per 10 kg soil (corresponding to 0; 2; 4; 6; 8 and 10 t ha^{-1} of MSWC, respectively)

Fig. 5 Dendrogram representing clustering of treatments based on different parameters for (A):TV1, (B): TV23

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Table 1 Selected physical and chemical properties of used soils 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 ± sample standard deviation). Values in parentheses indicate Legislation for MSWC addition in India [30]. Values in square brackets represent the percentages of each fraction with respect to total soil Cd.

Parameter	Soil	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)
TOC (%)	1.21±0.08	21.4±0.5 (>16.0)
WSC (%)	0.05±0.02	0.73±0.09
CEC ($cmol\ kg^{-1}$)	8.26±0.68	82.3±1.9 (≥60)
Total N (%)	0.15±0.03	1.56±0.08 (≥0.5)
P as P_2O_5†	9.26±0.40	2.82±0.14 (≥0.5)
K as K_2O††	91.3±8.3	14.9±1.4 (≥1.0)
Total heavy metals:		
Cd	0.02±0.01	2.44±0.77 (5)
Cr	1.25±0.08	9.00±0.04 (50)
Cu	9.58±0.03	61.7±0.4 (300)
Ni	1.82±0.03	8.26±0.23 (50)
Zn	67.4±2.3	283±4 (1000)
DTPA extractable heavy metals:		
Cd	BDL	0.66±0.08
Cr	0.08±0.003	1.00±0.06
Cu	8.27±0.02	9.81±0.19
Ni	0.96±0.002	1.06±0.06
Zn	29.5±0.01	62.4±0.7
Different fractions of Cd*:		
F1	BDL	0.09±0.04 [3.7]
F2	BDL	0.10±0.02 [4.1]
F3	BDL	0.41±0.06 [16.8]
F4	BDL	0.67±0.02 [27.5]
F5	0.007±0.001[35.0]	0.77±0.04 [31.6]
F6	0.013±0.004[65.0]	0.34±0.02 [13.9]
Germination index (%):		
Wheat (*Triticum aestivum* L.)	Not done	96.0±5.0
Indian mustard (*Brassica campestris* L)	Not done	98.3±5.0

BDL,below detection limits (detection limit of Cd in AAS is 0.001 $mg\ L^{-1}$)

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

† plant available P as $P_2O_5$for soil and total P as P_2O_5 for MSWC

†† exchangeable K as $K_2O$for soil and total K as K_2O for MSWC

Table 2 Selected physical and chemical properties of soil and fractionation (mg kg $^{-1}$) of Cd in soil after two years receiving MSWC treatments (values represent the mean of three replications ± sample standard deviation). Values in square brackets represent the percentages of each fraction with respect to total soil Cd

Parameters	Tea clone	Treatment[#]					
		T0	T1	T2	T3	T4	T5
pH (unit less)	TV1	[a]5.44±0.03	[a]5.33±0.01	[a]5.32±0.02	[a]5.39±0.02	[a]5.40±0.04	[a]5.42±0.03
	TV23	[a]5.41±0.01	[a]5.30±0.02	[a]5.38±0.02	[a]5.41±0.04	[a]5.42±0.03	[a]5.44±0.06
EC (dSm^{--1})	TV1	[a]0.06±0.02	[a]0.08±0.02	[a]0.08±0.02	[a]0.09±0.01	[a]0.09±0.01	[a]0.09±0.01
	TV23	[a]0.05±0.02	[a]0.06±0.02	[a]0.07±0.02	[a]0.11±0.03	[a]0.12±0.04	[a]0.12±0.03
Organic carbon (%)	TV1	[a]1.11±0.01	[a]1.18±0.02	[a]1.21±0.02	[a]1.23±0.03	[a]1.26±0.04	[a]1.29±0.05
	TV23	[a]1.13±0.01	[a]1.17±0.02	[a]1.18±0.02	[a]1.22±0.02	[a]1.24±0.02	[a]1.33±0.04
WSC(%)	TV1	[a]0.01±0.001	[a]0.04±0.001	[a]0.05±0.003	[a]0.06±0.001	[a]0.09±0.002	[a]0.11±0.003
	TV23	[a]0.02±0.003	[a]0.05±0.003	[a]0.10±0.002	[a]0.13±0.001	[a]0.14±0.004	[a]0.16±0.004
CEC (cmol kg^{--1})	TV1	[a]7.86±0.12	[a]8.31±0.11	[a]8.33±0.16	[a]8.33±0.24	[a]8.37±0.26	[a]8.41±0.18
	TV23	[a]7.81±0.21	[a]8.28±0.19	[a]8.32±0.17	[a]8.34±0.22	[a]8.39±0.21	[a]8.39±0.31
Total N (%)	TV1	[a]0.11±0.013	[a]0.16±0.011	[a,b]0.18±0.011	[a,b]0.19±0.012	[a,b]0.23±0.016	[b]0.23±0.011
	TV23	[a]0.12±0.016	[a]0.14±0.013	[a]0.17±0.015	[a,b]0.18±0.022	[a,b]0.22±0.023	[b]0.21±0.019
Total P as P_2O_5	TV1	[a]8.29±0.11	[a]8.89±0.09	[a]8.95±0.22	[a]8.99±0.34	[a]8.24±0.28	[a]8.74±0.32
	TV23	[a]8.05±0.26	[a]8.78±0.24	[a]8.90±0.21	[a]8.90±0.28	[a]8.98±0.22	[a]9.21±0.36
Total K as K_2O	TV1	[a]87.2±2.1	[a]94.2±2.1	[a]95.1±3.3	[a]95.3±5.0	[a]95.4±5.3	[a]95.4±3.5
	TV23	[a]94.2±2.2	[a]94.2±2.2	[a]95.3±2.9	[a]95.3±3.2	[a]95.4±2.3	[a]95.4±3.1
Total Cd[*]	TV1	[a]0.01±0.001	[b]0.14±0.002	[b]0.16±0.001	[b]0±0.003	[b,c]0.17±0.01	[c]0.20±0.001
	TV23	[a]0.01±0.001	[b]0.17±0.01	[b]0.16±0.004	[b]0.18±0.003	[b,c]0.18±0.01	[c]0.26±0.03
							Different fractions of Cd[‡]
F1	TV1	BDL[--]	BDL [--]	BDL[--]	BDL[--]	BDL[--]	BDL[--]
	TV23	BDL [--]	BDL [--]	BDL[--]	BDL[--]	BDL[--]	BDL[--]
F2	TV1	BDL [--]	BDL[--]	BDL[--]	[a]0.02±0.001[12.5]	[a]0.02±0.004[11.8]	[a]0.03±0.001[15.0]

	TV23	BDL [--]	BDL[--]	^{b}0.02±0.002[12.5]	^{b}0.02±0.004[12.5]	^{b,c}0.03±0.004[17.6]	^{c}0.04±0.002[20.0]
F3	TV1	BDL [--]	BDL[--]	^{b,c}0.02±0.002[12.5]	^{b,c}0.02±0.002[12.5]	^{b,c}0.02±0.001[11.8]	^{c}0.03±0.003[15.0]
	TV23	BDL [--]	BDL[--]	^{a}0.03±0.003[18.8]	^{a,b}0.03±0.004[18.8]	^{a,b}0.03±0.003[17.6]	^{b}0.04±0.01[20.0]
F4	TV1	BDL [--]	^{a}0.03±0.002[21.4]	^{a}0.03±0.002[18.8]	^{a}0.02±0.005[12.5]	^{a}0.03±0.002[17.6]	^{a}0.04±0.002[20.0]
	TV23	BDL [--]	^{a}0.04±0.004[28.6]	^{a}0.04±0.004[25.0]	^{a}0.04±0.006[25.0]	^{a,b}0.04±0.002[23.5]	^{b}0.05±0.002[25.0]
F5	TV1	BDL [--]	BDL [--]	^{b,c}0.02±0.003[12.5]	^{b,c}0.02±0.001[12.5]	^{b,c}0.02±0.004[11.8]	^{c}0.03±0.001[15.0]
	TV23	BDL[--]	^{b}0.02±0.002[14.3]	^{b,c}0.03±0.001[18.8]	^{b,c}0.03±0.002[18.8]	^{b,c}0.03±0.002[17.6]	^{c}0.03±0.002[15.0]
F6	TV1	BDL[--]	^{b}0.05±0.01[35.7]	^{b}0.06±0.004[37.5]	^{b,c}0.08±0.005[50.0]	^{b,c}0.08±0.008[47.1]	^{c}0.08±0.005[40.0]
	TV23	BDL[--]	^{b}0.06±0.01[42.9]	^{b}0.05±0.002[31.3]	^{b,c}0.06±0.002[37.5]	^{b,c}0.06±0.004[35.3]	^{c}0.08±0.007[40.0]

[#]T0: Control (without MSWC); T1:7.69 g MSWC; T2: 15.38 g MSWC; T3: 23.08 g MSWC; T4: 30.77 g MSWC; and T5: 38.46 g MSWC per pot with 10 kg soil (corresponding to 0, 2, 4, 6, 8, and 10 t ha^{-1} of MSWC, respectively)

[†]same symbol within column indicates there is no significant difference between treatments and different symbols indicate the pair of treatments are significantly different at 5 % level of significance).

BDL,below detection detectable limit (detection limit of Cd in AAS is 0.001 mg L^{-1})

[‡] F1: water-soluble; F2: exchangeable; F3: bound to carbonates; F4: bound to Fe and Mn; F5: organically bound; and F6: residual fractions

Table 3 Geoaccumulation index (I_{geo}) of cadmium metal at different MSWC treatments influenced by two popularly cultivated Tocklai vegetative clones (TV1 and TV23)

Clone	Treatment					
	T0	T1	T2	T3	T4	T5
	Value of geo-accumulation					
TV1	--1.74	2.23	2.41	2.41	2.51	2.74
	Class of I_{geo}					
	●	❸	❸	❸	❸	❸
TV23	--1.74	2.51	2.41	2.58	2.58	3.12
	Class of I_{geo}					
	●	❸	❸	❸	❸	❹

Class of geo-accumulation index (I_{geo})*						
0 (Practically uncontaminated)	1 (Uncontaminated to moderately contaminated)	2 (Moderately contaminated)	3 (Moderately to heavily contaminated)	4 (Heavily contaminated)	5 (Heavily to extremely contaminated)	6 (Extremely contaminated)
Range of I_{geo}						
$I_{geo} \leq 0$	$0 < I_{geo} < 1$	$1 < I_{geo} < 2$	$2 < I_{geo} < 3$	$3 < I_{geo} < 4$	$4 < I_{geo} < 5$	$I_{geo} > 5$
Legend						
●	❶	❷	❸	❹	❺	❻

*source: [9]

Table 4. Pearson's correlation coefficients between biomass and other variables in TV1 and TV23 along with their statistical significance

Parameter	Biomass			
	Main root	Feeder root	Stem	Leaf
		Clone: TV1		
Cd in main root	0.933**	0.827*	0.642	0.971**
Cd in feeder root	0.957**	0.851*	0.627	0.959**
Cd in stem	0.892*	0.883*	0.684	0.947**
F2	0.692	0.862*	0.856*	0.912*
F3	0.786	0.953**	0.911*	0.895*
F4	0.830*	0.956**	0.892*	0.896*
F5	0.732	0.759	0.832*	0.777
F6	0.656	0.910*	0.935**	0.848*
RAC	0.869*	0.967**	0.743	0.889*
I_{geo}	0.801	0.944**	0.896*	0.900*
TF in main root	0.886*	0.979**	0.818*	0.891*
TF in feeder root	0.872*	0.989**	0.820*	0.856*
TF in stem	0.845*	0.949**	0.870*	0.921**
		Clone: TV23		
Cd in main root	0.830*	0.771	0.887*	0.847*
Cd in feeder root	0.811*	0.742	0.811*	0.823*
Cd in stem	0.759	0.596	0.738	0.692
F2	0.846*	0.725	0.906*	0.798
F3	0.916*	0.770	0.901*	0.852*
F4	0.917*	0.766	0.890*	0.852*
F5	0.899*	0.663	0.845*	0.753
F6	0.853*	0.643	0.884*	0.742
RAC	0.935**	0.807	0.949**	0.890*
I_{geo}	0.925**	0.762	0.922**	0.848*
TF in main root	0.875*	0.593	0.870*	0.706
TF in feeder root	0.853*	0.745	0.882*	0.829*
TF in stem	0.860*	0.653	0.893*	0.751

*and ** denote significant difference at 5% and 1% level, respectively.

Table 5. Stepwise regression analysis (DF = degrees of freedom)

TV1			
Dependent variable: Stem biomass, R^2=0.894			
Variable	Parameter Estimate	Standard Error	Probability
Intercept	17.9506	10.3792	0.1822

F2	3001.5	315.277	0.0025
F5	--1240.9	220.356	0.0111
Cd in Stem	--165.41	31.6042	0.0136
ST/FR	37.2962	18.92639	0.1434
T_i	--392.277	71.39775	0.0119
Dependent variable: Leaf biomass, R^2=0.898			
Intercept	22.8157	12.7109	0.1228
Cd in feeder root	29.4302	9.01537	0.0172
ST/FR	--25.507	11.123	0.0617
Dependent variable: Main root biomass, R^2=0.852			
Intercept	19.98	0.29063	0.0093
F4	2906.84	16.1132	0.0035
F5	--1326.7	35.511	0.017
Total Cd	--252.45	5.50802	0.0139
RAC	0.69946	0.00791	0.0072
Cd in stem	--268.07	2.59767	0.0062
T_i	1552.791	9.53177	0.0039
Dependent variable: Feeder root biomass, R^2=0.957			
Intercept	2.71047	0.54737	0.0043
F5	97.0684	22.3504	0.0074
Cd in feeder root	--17.52	2.8185	0.0016
T_i	119.295	14.9964	0.0005
TV23			
Dependent variable: Stem Biomass, R^2=0.965			
Intercept	214.743	40.9447	0.0002
RAC	--1.0424	0.23306	0.0008
Cd in main root	--72.518	18.4938	0.002
ST/FR	--170.63	33.8955	0.0003
T_i	16.2347	1.06831	<0.0001
Conc_Cd	381.23	187.926	0.0653
Dependent variable: Leaf Biomass, R^2=0. 791			
Intercept	--1.5108	2.25998	0.5133
T_i	8.95462	1.14891	<0.0001
Dependent variable: Main root Biomass, R^2=0.879			
Intercept	--1.4476	1.97542	0.4743
T_i	9.79279	1.00425	<0.0001
Dependent variable: Feeder root Biomass, R^2=0.705			
Intercept	--18.821	11.9601	0.1364
ST/FR	19.3202	12.3127	0.1375

T_i	2.27056	0.37845	<0.0001