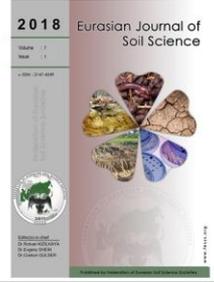




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Trace minerals pollution in south indian branded tea, and from the tea waste disposal sites

Selvam Ramarajan, Sugumar Susila, Duraisamy Tamilselvi,
Muthunarayanan Vasanthi *

Department of Environmental Biotechnology, School of Environmental Sciences, Bharathidasan University,
Tiruchirappalli, India

Abstract

We aim to quantify heavy metals in tea powders (packed and used), tea waste disposal sites and in the soil away from the site of disposal and to isolate the bacteria from both the soil sample. Atomic Absorption Spectrometer (AAS) analysis revealed chromium in the samples collected from Thanjavur (0.12 mg/kg) and Tiruchirappalli (4.77 mg/kg). The quantity of Copper was between 0.14 mg/kg and 0.97 mg/kg Namakkal and Erode samples respectively. We also found trace levels of other heavy metals. The spatial map distribution patterns of selected Physico-chemical parameters like pH, EC, Alkalinity, Total carbon in the disposal sites were presented. Some of the metal-intolerant strains isolated from the disposal sites became resistant. Further investigation would unravel the possible mechanism behind Chromium reduction by the selected isolates.

Keywords: Toxic metal, tea powder, AAS, spatial map, chromium, biosorption.

Article Info

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Introduction

Tea from the leaves of the shrub *Camellia sinensis* is the most preferred consumable beverage worldwide (Fernández-Cáceres et al., 2001). Approximately about 18-20 billion consumers drink tea daily. Despite its medicinal properties, it also contains numerous toxic elements (Fe, Mn, Cr, Zn, Cu) (TEs) in trace quantity (Shen and Chen, 2008). These TEs are present in domestic and industrial wastewater, dust, vehicular exhaust and industrial emissions etc. that affect agricultural lands and plants (Singh et al., 2010; Muntean et al., 2013). The TEs such as cadmium from fallouts of vehicular exhaust and dust were reported to affect tea plants by getting deposited on the leaves. Other TEs such as Mercury, Lead, Arsenic, Chromium, Iron and Cadmium were also found in tea leaves in trace level, however harmful to humans (Wang et al., 2008; Han et al., 2006; Sadeghi et al., 2011). Studies exist to demonstrate the ROS production along with oxidative stress play a major role in the toxicity and carcinogenicity of metals such as As, Cd, Cr and Hg (Tchounwou et al., 2001; Tchounwou et al., 2004; Yedjou and Tchounwou, 2006; Yedjou and Tchounwou, 2007). Due to their toxicity these heavy metals were ranked as priority metals that are of great public health significance (Tchounwou et al., 2012). The occurrence of TEs in tea plants is mainly by absorption from nutrient supplementation, growth media, industrial wastes, organic and inorganic fertilizers, and pesticides. These TEs cause delayed flowering, reduced chlorophyll content and shortening the shoot length (Mani et al., 2014). Chromium is reported to be one of the most popular TEs and is found to get added tea mainly from the crush-tear-curl rollers used to prepare tea which usually contain chromium (Seenivasan et al., 2008). No

* Corresponding author.

Department of Environmental Biotechnology, School of Environmental Sciences, Bharathidasan University, Tiruchirappalli 620 024, India

Tel.: +91431 2407088

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E-mail address: drvasanthi02@gmail.com

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studies were performed hitherto on the estimation of toxic metals in tea powder and tea waste disposal sites, however these types of agro based waste has been used as adsorbents for heavy metal removal (Demirbas, 2008). Hence the present study has been framed with the following objectives.

The objective were to,

- (i) analyze TEs in commercially available packed Tea Powder (TP), Used Tea Powder (UTP), soil samples collected from the tea waste disposal site (Soil Contaminated- SC) and the soil samples collected away from the disposal site (Soil Un-Contaminated- SUC).
- (ii) Isolate the soil bacteria collected from disposal sites (SC and SUC)
- (iii) Evaluate the metal resistance potential of the isolated bacterial strains against chromium and to determine MTC (Maximum Tolerance Concentration) of individual bacterial strains, and
- (iv) To determine the physico-chemical characteristics of the soil with spatial map presentation.

Material and Methods

Sample collection

The soil samples were collected from various districts of Tamilnadu (South India) such as Chennai, Kanyakumari, Tiruchirappalli, Salem, Namakkal, Thiruvarur, Thanjavur, Pudukkottai, Madurai and Erode (Figure 1). We made a V-shaped cut (15-20 cm depth) and collected smooth side of the soil (1 cm slice) in a clean bucket and mixed well (Figure 2). The tools used for sampling includes shovel, spade and augers (Parker, 2009). The samples were homogenized, air-dried and sieved with a 2 mm wire mesh before analysis.



Figure 1. Study area- Sampling sites

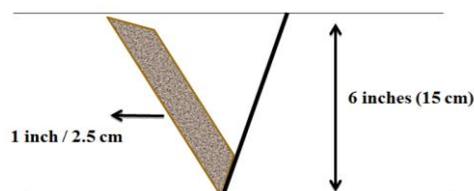


Figure 2. Sample collection method

Preparation of soil sample for Physico-chemical analysis

The preparation of soil sample is, 1 g of soil sample was dissolved in 20 mL of double distilled water and kept for 30 min in shaker. After mixing, the sample were filtered and subjected to the analysis of Physico-chemical parameters such as pH, electrical conductivity (EC), alkalinity and organic carbon (without water mixing) (APHA, 2005).

$$I = \sqrt{X1 = X2 = X3 = X4 = X5 / 5}$$

Note: This formula represented the spatial distribution of geographical map with weightage data included physico-chemical and Heavy metal analysis. (I=Index, X=Weightage of metals impact)

Quantification of Heavy metals in the samples

To quantify the heavy metals, 1 g of TP, UTP and 0.5 g of SC, SUC were digested using Microwave digester (MDS-6, SINEO Microwave Chemistry Technology Co., Ltd). The operating condition specific for each metals are summarized in Table 2, 3 and 4 (Xin et al, 2010). Samples were analyzed in triplicate using Graphite Furnace Atomic Absorption Spectrophotometer (Thermo Scientific ICE 3000 series, Solar data station (v.11.02)) to quantify Fe, Mn, Cr, Zn, and Cu.

Biosorption studies

The bacterial strains isolated from disposal sites (SC and SUC) were streaked onto the nutrient agar media supplemented with metal concentrations ranging from 100 to 1000 ppm. For biosorption studies, the plates were incubated at 30°C for 24 to 72 hours and the isolated bacterial strains were inoculated into 100 mL of nutrient medium supplemented with glucose (1%) and urea (1%) containing different concentrations of Cr (VI) as K₂Cr₂O₇ (100–500 ppm) followed by incubation at 37°C for 120 rpm for 24 hours in a mechanical shaker. The samples were centrifuged at 10,000 rpm for 15 min in a cooling centrifuge (Model: REMI C-24) at 4°C and the supernatant was subjected to ultra-sonication and chromium concentration was determined using di-phenyl carbazide reagent in acid solution (APHA, 1998) by measuring the absorbance at 540 nm in a spectrophotometer (2202- PC, Systronic). Thus Cr (VI)-reduction by the isolated strains was investigated. Samples were analyzed for the reduction of Cr (VI) at regular intervals.

Results and Discussion

The physico- chemical parameters such as pH, EC, alkalinity and total organic carbon were analyzed in used tea powder SC and SUC (Table 1) with representation of corresponding spatial map (Figure 3).

Table 1. Physico-chemical characteristics of tea waste SC and SUC: [Mean ±SD (N=3)].

Area of sample collection	Types of soil	pH	EC (µs/cm)	Alkalinity (mg/kg)	Carbon (mg/kg)
Tiruchirappalli	SC	5.183±0.015	0.866±0.014	33.33±5.773	5.486±0.195
	SUC	5.896±1.103	0.789±0.007	46.66±15.275	4.286±0.145
Salem	SC	8.066±0.055	0.616±0.007	46.66±05.773	2.613±0.208
	SUC	7.826±7.826	0.595±0.595	46.66±46.660	2.830±2.830
Namakkal	SC	7.813±0.051	0.587±0.004	80.00±10.000	1.720±0.206
	SUC	7.523±0.092	0.491±0.003	53.33±05.773	1.923±0.049
Chennai	SC	7.130±0.050	0.977±0.018	73.33±05.773	1.876±0.051
	SUC	7.156±0.020	0.818±0.002	96.66±5.773	2.946±0.020
Thanjavur	SC	6.146±0.404	0.639±0.005	46.66±10.000	2.333±0.055
	SUC	7.186±0.070	0.167±0.028	76.66±05.773	2.846±0.120
Thiruvarur	SC	6.833±0.404	0.153±0.005	50.00±10.000	2.943±0.055
	SUC	7.186±0.070	0.167±0.028	76.66±05.773	2.846±0.120
Madurai	SC	8.003±0.060	0.743±0.022	50.00±10.000	3.323±0.222
	SUC	7.386±0.180	0.448±0.022	70.00±10.000	1.653±0.150
Erode	SC	6.560±0.245	0.754±0.012	43.33±05.773	1.676±0.090
	SUC	7.036±0.572	0.806±0.032	60.00±10.000	1.913±0.120
Kanyakumari	SC	7.363±0.245	0.169±0.010	40.00±10.000	1.800±0.170
	SUC	6.793±0.410	0.267±0.219	43.33±05.773	1.897±0.068
Pudukkottai	SC	7.403±0.218	1.000±0.105	73.34±11.547	2.423±0.090
	SUC	6.556±0.196	0.620±0.404	73.33±05.773	1.913±0.058

Table 2. The acid composition of Microwave digester mixture.

Sample type	Gram (g)	HNO ₃ (65%)	H ₂ O ₂ (mL)	Time (min)
TP	1.0	6 mL	2.5	20
UTP	1.0	6 mL	2.5	20
SC	0.5	4 mL	6	30
SUC	0.5	4 mL	6	30

Table 3. Operating conditions with wavelength for GF-AAS.

Elements	Mn	Fe	Cr	Zn	Cu
Sampling volume	20	20	20	20	20
Wavelength (nm)	259.4	269.4	215.9	213.2	326.7
Lamp current (mA)	8	6	10	13	12
Gas flow (Inner /Outer)	1.5	1.5	1.5	1.5	1.5
Band Pass width	0.6	0.5	0.4	0.3	0.3

Table 4. Atomization temperature–time program

	Temp. °C	Ramp (°C s ⁻¹)	Hold (s)	Purge Argon flow
Drying	110	50	20	2
Pyrolysis I	600	30	10	2
Pyrolysis II	800	20	5	2
Atomization	2075	-	3	0
Cleaning	2300	-	2	2

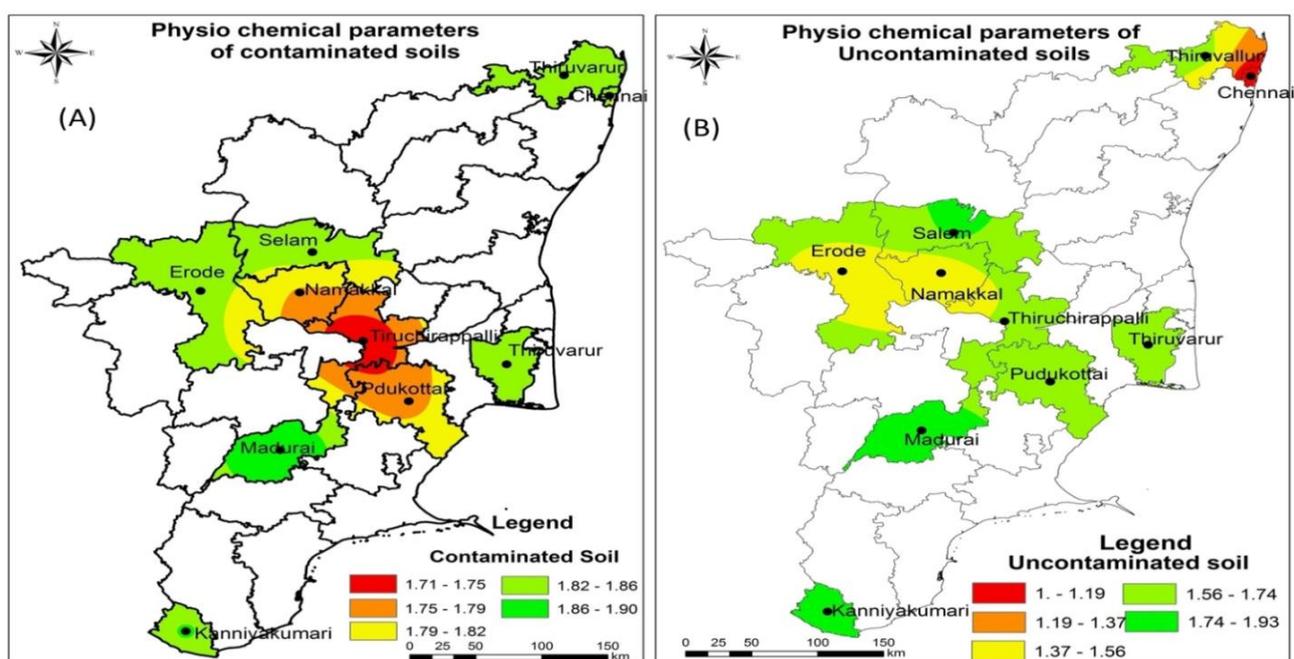


Figure 3. Spatial distribution of monitoring stations across Tamilnadu and this map shows the physicochemical (pH, EC, Alkalinity, Carbon) parameters. The picture named as (A). SC (B). SUC

The concentration of TEs from SC and SUC was determined. The quantity of Fe was 1.01 ppm in the samples collected at Salem and Thanjavur samples contained 13.27 ppm, which was the maximum in SC and 3.20 ppm in SUC. Zn was found to be in the range of 0.22 ppm to 3.29 ppm in SC, and the highest quantity was in Madurai and Namakkal samples and 0.85 ppm in SUC. The minimum concentration of Cr was 0.12 ppm, and as high as 4.77 ppm in Tiruchirappalli (SC) samples and 4.07 ppm in SUC. The concentration of Cu in SC was less (0.15 ppm) in Namakkal samples and it was higher in Chennai samples (1.386 ppm). The concentration of Cu in SUC was about 1.046 ppm in samples collected from Kanyakumari and it was 0.076 ppm in SUC collected from Tiruchirappalli. The least quantity of Mn (0.56 ppm) was in Namakkal samples, whereas the highest quantity (14.67 ppm) was present in Tiruchirappalli (SC) samples and 12.48 ppm in SUC. Further, the changes in the concentration of all heavy metals varied significantly between different districts (Figure 4).

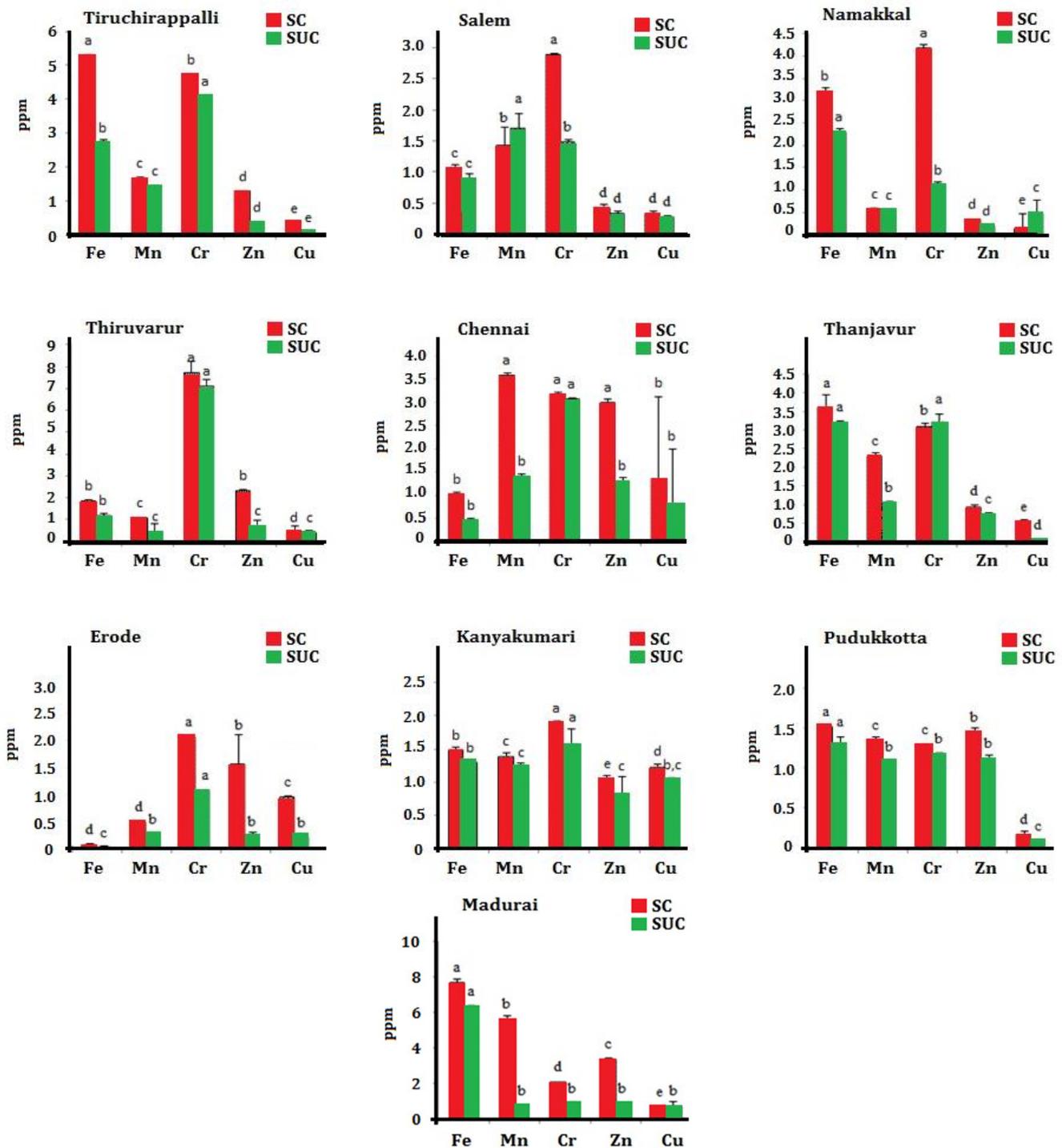


Figure 4. Changes of the heavy metals (Fe, Mn, Cr, Zn, Cu) concentration in soil contaminated (SC) and soil uncontaminated (SUC) using Atomic adsorption spectrophotometer values were expressed and Mean \pm SD (N=3). Bar with different alphabets represents they are significantly different from each other and those with same alphabets have insignificant changes ($P < 0.05$).

The presence of TEs in the soil samples and different tea powder samples was further represented as spatial maps. The color range (TEs-SC) of 1.34 – 1.48 indicated the areas with maximum contamination, followed by 1.48–1.62, which indicated higher contamination. The range of 1.62–1.76 represented moderate contamination, 1.76–1.90 indicated low level of contamination, and 1.90–2.04 revealed the least contamination. The spatial map of SUC showed that the range of TEs 1.48–1.58 exhibited highest contamination followed by 1.58 – 1.69 that indicated higher contamination. Subsequent moderate and lower contamination were indicated by the range of 1.69–1.79 and 1.79–1.89, respectively. The lowest level of contamination falls in the color range of 1.89 – 2.0 (Figure 5).

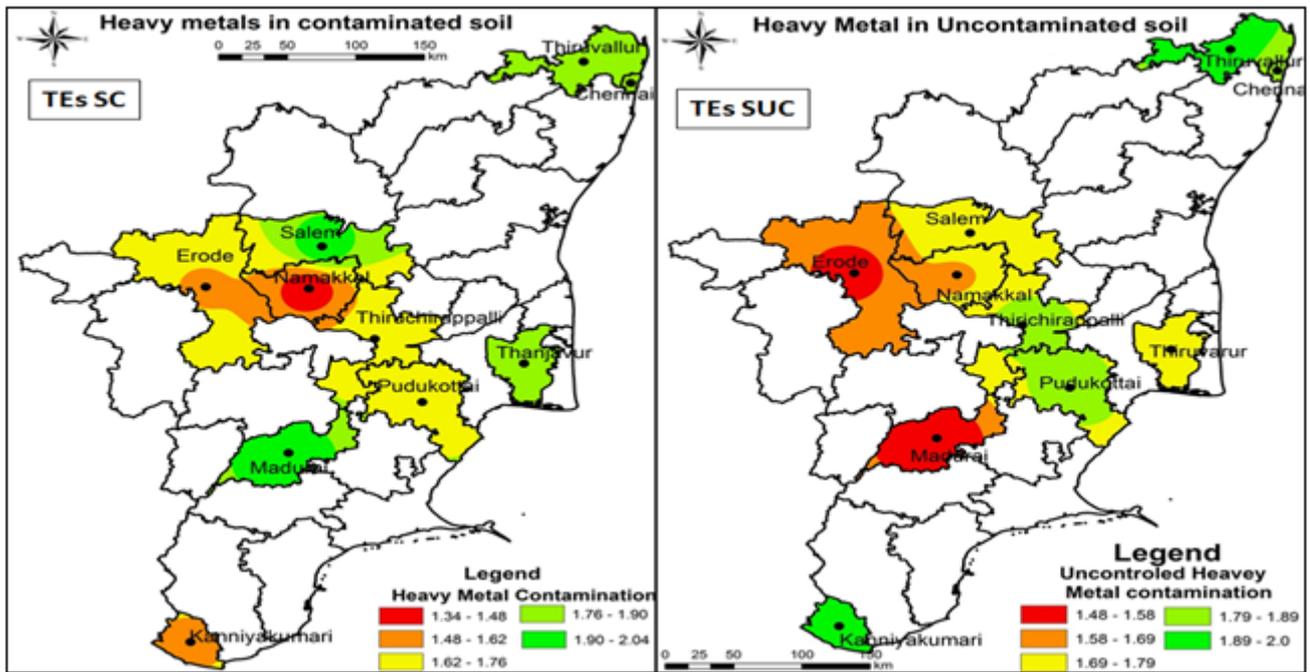


Figure 5. Spatial distribution and concentration of heavy metals in the study area TES-SC and TES-SUC.

TEs content was also analyzed in packed and used-tea powders. The concentration of Fe was 0.0011 ppm in Brand x3 as the minimum and the maximum was about found as 0.0128 ppm in Brand x7. Zn concentration was 0.0138 ppm in Brand x4 and in Brand x6 it was 0.0447 ppm. Cr was found at minimum concentration (0.0348 ppm) in Brand x7 and the maximum (0.0742 ppm) was found in in Brand x1. Cu in Brand x5 was 0.0189 ppm and in Brand x1 it was 0.0448 ppm. Mn content was 0.56 ppm, and 0.067 ppm in Brand x6 and Brand x7, respectively (Figure 6).

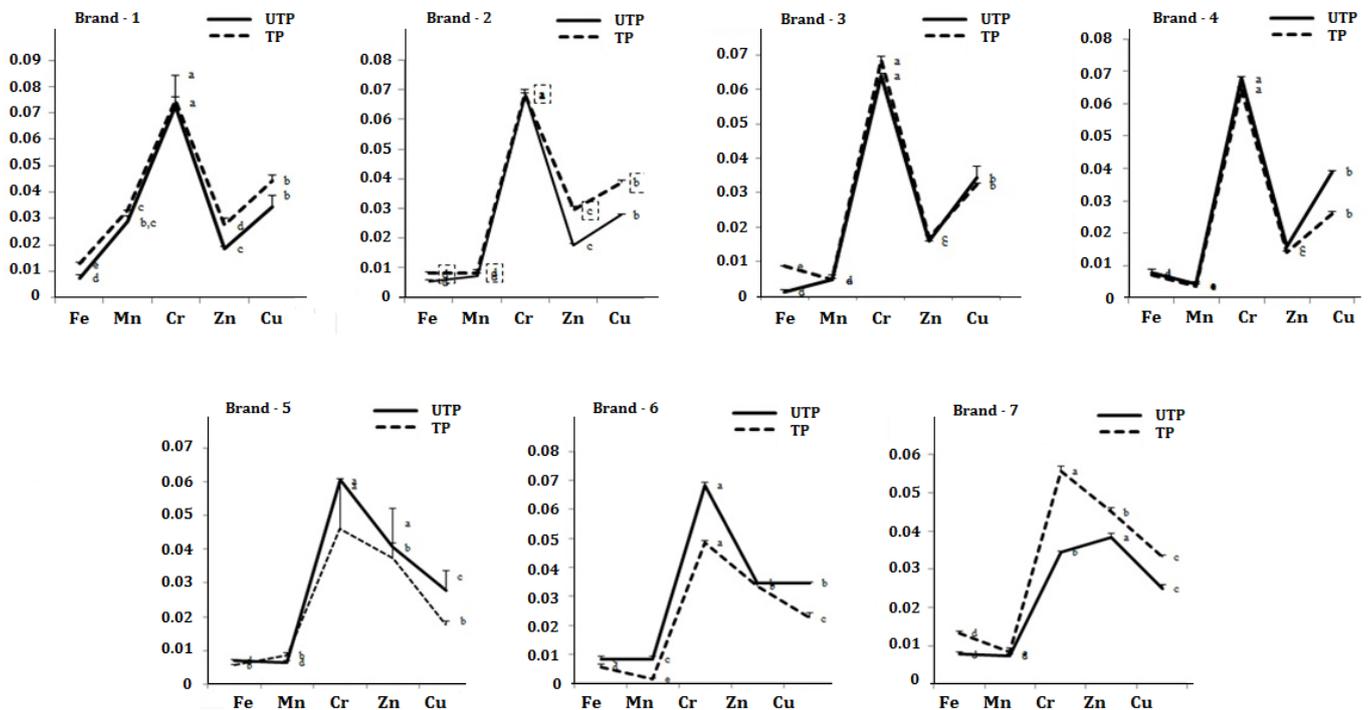


Figure 6. Changes in the TEs (Fe, Mn, Cr, Zn, Cu) concentration in Tea Powder (TP) and Used Tea Powder (UTP) using Atomic Adsorption Spectrophotometer. Values were expressed as Mean \pm SD (N=3) Bar with different alphabets represents they are significantly different from each other and those with same alphabets have insignificant changes ($P \leq 0.05$) and the unit of TEs concentration is expressed as ppm.

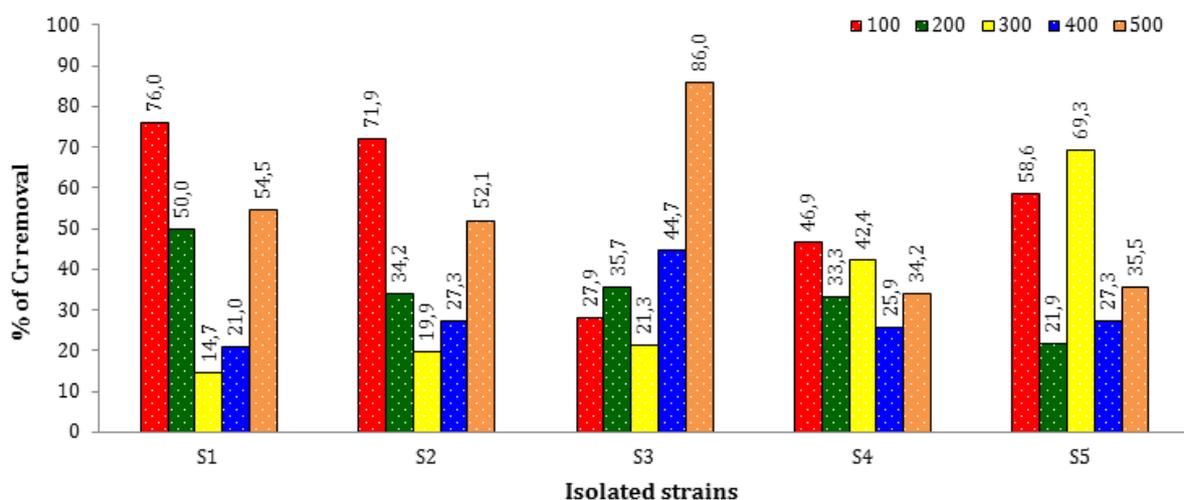
The MTCs (Maximum tolerable concentration) of the bacterial strains present in various natural habitats such as soil, water, sediments and sewage amended soil have been studied (Abbas and Edwards, 1989). The

majority of microbes were found to possess two types of uptake mechanisms to reduce the cations and anions. First one is fast, unspecific, constitutively expressed and obsessed by the chemi osmotic gradient across the cytoplasm covering of bacteria. The second one is inducible, possesses high substrate specificity and is slower, repeatedly uses ATP hydrolysis as the power supply and is only produced by the cell in times of need and under special metabolic conditions. Higher amount of essential and nonessential metals can affect the cell membranes; modify enzyme specificity; interrupt cellular functions; and damage the structure of DNA and impose oxidative stress on microbes too (Bruins et al., 2000). In this paper, the metal tolerant microbial strains were randomly selected for their level of resistance against Cr (VI) concentration. From the MTC analysis, a significant difference in the resistance levels of the bacterial strains isolated from both SC and SUC was noticed (Table 5). The MTC of all isolates *Bacillus sp* (S1), *Bacillus megaterium* (S2) (isolates collected from SC), (S3) and *Pasteurella haemolytica* (S4) (isolates from SUC) was 700 ppm. Altogether, all the isolates exhibited similar, tolerance against chromium. The reduction level was 76% in 100 ppm by strain 1, 50.01% and 69.30% in 300 ppm by strain S5, and 44.70% in 400ppm and 86.04% 500ppm by strain S3 (Figure 7).

Table 5. Cr tolerance of the isolated bacterial strains.

Isolated strains	Activity of isolated bacteria in different concentration (ppm) of Cr(VI)									
	100	200	300	400	500	600	700	800	900	1000
S1	R	R	R	R	R	R	R	S	A	A
S2	R	R	R	R	R	R	S	A	A	A
S3	R	R	R	R	R	R	S	A	A	A
S4	R	R	R	R	R	R	S	A	A	A

*R- Resistance; A- Absence of growth; S- Sensitive



*(S1-S4 refers to the isolated bacterial strains).

Figure 7. Cr reduction by isolated bacterial strains

Already reports exist to prove that the metal biosorption is a two-step process where the functional groups of the metal ions (phosphate, hydroxyl, carboxyl, amino, sulphide, sulphur group, etc.) gets adsorbed to the surface of cells by interaction. However, reports also suggest that TEs can also bind on the surface, which can be eventually eluted by other ions present in chelating agent or acid. Hence this work on identification and quantification of the metal ions in the soil samples is a need of the hour. Further work is needed to understand the mechanism of Biosorption of the metal ions by the autochthonous organisms present in the contaminated areas. Similarly, biosorption referring to the penetration of metal ions into the cell membrane has been already reported (Veglio and Beolchini, 1997; Madrid and Cámara, 1997). Studies exist to understand the biosorption of Cr (VI) and Fe (III) by *Streptococcus equisimilis*, *S. cerevisiae* and *Aspergillus niger* too (Goyal et al., 2003).

Conclusion

The heavy metal namely, Cr was present in higher quantities of TEs in used tea powder, which was about 0.0742 ppm. This data gives a clear understanding that certain quantity of heavy metal added to the soil and the soil had appreciable quantity of native heavy metals already; this may pose a threat to the environment

and disturb the normal flora present in the disposal site. Further investigation is needed to explore the possible mechanism behind chromium reduction by the selected strains. This study thus focuses on some branded tea, tea waste and disposal site as sources of heavy metal contamination and recommends that the disposal of such used tea waste must not be done in pristine environment so as to conserve the same.

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References

- Abbas, A., Edwards, C., 1989. Effects of metals on a range of *Streptomyces* species. *Applied and Environmental Microbiology* 55(8): 2030-2035.
- APHA, 2005. Standard methods for the examination of water and wastewater (Vol. 2). American Public Health Association, Washington, USA.
- Bruins, M.R., Kapil, S., Oehme, F.W., 2000. Microbial resistance to metals in the environment. *Ecotoxicology and Environmental Safety* 45(3): 198-207.
- Demirbas, A., 2008. Heavy metal adsorption onto agro-based waste materials: A review. *Journal of Hazardous Materials* 157(2-3): 220-229.
- Fernández-Cáceres, P.L., Martín, M.J., Pablos, F., González, A.G., 2001. Differentiation of tea (*Camellia sinensis*) varieties and their geographical origin according to their metal content. *Journal of Agricultural and Food Chemistry* 49(10): 4775-4779.
- Goyal, N., Jain, S.C., Banerjee, U.C., 2003. Comparative studies on the microbial adsorption of heavy metals. *Advances in Environmental Research* 7(2): 311-319.
- Han, W.Y., Zhao, F.J., Shi, Y.Z., Ma, L.F., Ruan, J.Y., 2006. Scale and causes of lead contamination in Chinese tea. *Environmental Pollution* 139(1): 125-132.
- Madrid, Y.i Cámara, C., 1997. Biological substrates for metal preconcentration and speciation. *TrAC Trends in Analytical Chemistry* 16(1): 36-44.
- Mani, D., Kumar, C., 2014. Biotechnological advances in bioremediation of heavy metals contaminated ecosystems: an overview with special reference to phytoremediation. *International Journal of Environmental Science and Technology* 11(3): 843-872.
- Muntean, N., Muntean, E., Creta, C., Duda, M., 2013. Heavy metals in some commercial herbal teas. *ProEnvironment* 6: 591-594.
- Parker, R., 2009. Plant & Soil Science: Fundamentals & Applications. Delmar Cengage Learning, New York, USA. 779p.
- Sadeghi, O., Tavassoli, N., Amini, M.M., Ebrahimzadeh, H., Daei, N., 2011. Pyridine-functionalized mesoporous silica as an adsorbent material for the determination of nickel and lead in vegetables grown in close proximity by electrothermal atomic adsorption spectroscopy. *Food Chemistry* 127(1): 364-368.
- Seenivasan, S., Manikandan, N., Muraleedharan, N.N., Selvasundaram, R., 2008. Heavy metal content of black teas from south India. *Food Control* 19(8): 746-749.
- Shen, F.M., Chen, H.W., 2008. Element composition of tea leaves and tea infusions and its impact on health. *Bulletin of Environmental Contamination and Toxicology* 80(3): 300-304.
- Singh, A., Sharma, R.K., Agrawal, M., Marshall, F.M., 2010. Risk assessment of heavy metal toxicity through contaminated vegetables from waste water irrigated area of Varanasi, India. *Tropical Ecology* 51(2): 375-387.
- Tchounwou, P.B., Centeno, J.A., Patlolla, A.K., 2004. Arsenic toxicity, mutagenesis, and carcinogenesis—a health risk assessment and management approach. *Molecular and Cellular Biochemistry* 255(1-2): 47-55.
- Tchounwou, P.B., Yedjou, C.G., Patlolla, A.K., Sutton, D.J., 2012. Heavy metal toxicity and the environment. In: Molecular, clinical and environmental toxicology, Volume 3 Environmental Toxicology. Luch, A. (Ed.). Springer, New York, USA. pp. 133-164.
- Veglio, F., Beolchini, F., 1997. Removal of metals by biosorption: a review. *Hydrometallurgy* 44(3): 301-316.
- Wang, X.P., Ma, Y.J., Xu, Y.C., 2008. Studies on contents of arsenic, selenium, mercury and bismuth in tea samples collected from different regions by atomic fluorescence spectrometry. *Guang pu xue yu guang pu fen xi= Guang pu* 28(7): 1653-1657. [in Chinese]
- Xin, J., Huang, B., Yang, Z., Yuan, J., Dai, H., Qiu, Q., 2010. Responses of different water spinach cultivars and their hybrid to Cd, Pb and Cd–Pb exposures. *Journal of Hazardous Materials* 175(1): 468-476.
- Yedjou, C.G. and Tchounwou, P.B., 2006. Oxidative stress in human leukemia (HL-60), human liver carcinoma (HepG2), and human (Jurkat-T) cells exposed to arsenic trioxide. Proceedings of the 9th International Symposium on Metal Ions in Biology and Medicine Held. Lisboa, Portugal. 21-24 May 2006. pp. 298.
- Yedjou, C.G., Tchounwou, P.B., 2007. In-vitro cytotoxic and genotoxic effects of arsenic trioxide on human leukemia (HL-60) cells using the MTT and alkaline single cell gel electrophoresis (Comet) assays. *Molecular and Cellular Biochemistry* 301(1-2): 123-130.