Malaysian Journal of Microbiology, Vol 16(2) 2020, pp. xxx-xxx
DOI: http://dx.doi.org/10.21161/mjm.190519

Malaysian Journal of Microbiology
Published by Malaysian Society for Microbiology
(In SCOPUS since 2011)

Evaluation of the physio-chemical properties and microbial load of the soil polluted with coffee processing wastes

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Received 5 July 2019; Received in revised form 28 August 2019; Accepted 20 September 2019

ABSTRACT

Aim: The study was designed to evaluate the physio-chemical properties and microbial load of the soil polluted with coffee processing wastes such as coffee husk and coffee pulp.
Methodology and results: A total of ten soil samples were taken from three taluks of Coorg district of Karnataka, India. Out of which five soil samples were taken from places where the coffee processing wastes were dumped as landfills. The other five soil samples were taken from places free from coffee processing wastes which represent the control soil samples. The physical and chemical properties of the soil were measured using standard protocols. The highlight of the study was quantification of chemicals of ecotoxicological concern such as caffeine, polyphenols and tannin in soil samples. The identification and enumeration of soil bacteria, fungi, actinomycetes, yeast and plant growth promoting microorganisms were also done. The pollution with the coffee processing wastes make the soil acidic. The concentration of chemicals of ecotoxicological concern such as caffeine, polyphenols and tannins were significantly high in polluted soil. The colony forming units of plant growth promoting microorganism were declined significantly in the polluted soil. Instead of all these detrimental factors, the organic carbon, nitrogen, potassium, phosphorus and micronutrient content of the polluted soil was significantly high.
Conclusion, significance and impact of study: This study revealed the fact that the unscientific disposal of coffee processing wastes as landfill make the soil less fertile, damage the normal microbial diversity of the soil and would cause severe pollution problems.

Keywords: Coffee processing wastes, caffeine, polyphenols, tannin

INTRODUCTION

Coffee is considered as the second most consumed non-alcoholic beverage immediately after water (Ghosh and Venkatachalapathy, 2014). Around 400 billion cups of coffee are consumed by the world population annually (Teketay, 1999). Coffee is an economic crop and it stood the second most traded commodity next to petroleum products (Damatta *et al.*, 2008). As the world population increases, the demand for coffee also will increase (Gurram *et al.*, 2016). Due to the huge demand of coffee it is produced in a sustainable manner. More than 20 million people are working in this field. According to the International Coffee Organization, the average production worldwide for the last ten years (2008-2018) was found to be 143.54 million 60 kg of bag. Even though more than 80 coffee species have been identified around the world, only two are economically important. They are *Coffea arabica* (Arabica) and *Coffea canephora* (Robusta). World's largest coffee producers are Brazil (Fan *et al.*, 2000) accounting one-third of the total production. India remained in the 7th position producing more Robusta than Arabica.

Coorg (Kodagu) "the coffee land of India" is well known for its production and export of coffee. It is also referred as the "Switzerland of India" occupying the eastern and western slopes of the Western Ghats, has the coffee planted area of 107,089 hectares. According to the statistics from database on coffee by the Coffee Board of India, Coorg produced 116,550 Metric Ton (MT) of coffee during 2017-2018. After harvesting the coffee fruit undergo either wet or dry processing, producing coffee pulp and coffee husk respectively as wastes (Mazzafera, 2002). For every two tons of coffee processed by wet procedure, one ton of pulp is produced and for each ton of coffee processed by dry method, 0.18 ton of coffee husk is produced (Padmapriya *et al.*, 2013). In Indian scenario Arabica undergo wet processing and Robusta undergo dry processing. Thus, in Coorg

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ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 16(2) 2020, pp. xxx-xxx
DOI: http://dx.doi.org/10.21161/mjm.190519

approximately 9,949.6 MT of coffee processing wastes was generated during 2017-2018.

The disposal of coffee processing wastes such as coffee husk and coffee pulp is a major issue in Coorg region of Karnataka. The easiest way of disposal of the waste is to dump it directly to soil as landfills (Supplementary Figure 1) and it is most commonly practiced in Coorg. Both coffee husk and coffee pulp contain significant quantities of caffeine, tannins and polyphenols (Pandey *et al.*, 2000; Murthy and Naidu, 2012; Janissen and Huynh, 2018). These compounds are of toxic nature and the direct dumping of these wastes as landfills may cause environmental problems. The present study has been taken to evaluate the physio-chemical properties and the microbial load of the soil polluted with coffee processing wastes. This is the first report on the caffeine, polyphenols and tannin leachate to the soil polluted with coffee processing wastes.

MATERIALS AND METHODS

Collection of soil samples

A total of ten soil samples were taken from three taluks of Coorg district of Karnataka, India. Out of which five soil samples were taken from places where the coffee processing wastes were dumped as landfills (Ap, Bp, Cp, Dp, Ep). The other five soil samples were taken from places free from coffee processing wastes which represent the control samples (Ac, Bc, Cc, Dc, Ec). (Supplementary Figure 2, Supplementary Figure 3). The latitude and longitude of the sampling sites were presented (Supplementary Table 1).

Chemical analysis

Quantification of chemicals such as chlorogenic acid, caffeine and tannins were done. Chlorogenic acid concentration was measured quantitatively using Folin-Ciocalteu reagent (Sigma), (Azlim Almey *et al.*, 2010; Kamath *et al.*, 2015). Quantitative estimation of caffeine was made using the standard protocol with appropriate modifications for soil sample (Williamson, 1992). The tannins in the soil was quantified using Folin-Denis reagent (Sigma). (Pelozo *et al.*, 2008). UV-Visible Spectrophotometer (Thermo scientific) was used for determining the optical density (O.D) and there by the concentration of chlorogenic acid, caffeine and tannins were determined. The pH and electrical conductivity (E.C) of the soil samples were determined using multi-parameter tester 35 series (Thermo fisher Scientific). Total organic carbon (O.C) and Total Kjeldhal nitrogen were estimated by Walkley and Black rapid titration method (Walkley and Black, 1934) and micro Kjeldhal method (Singh and Pradhan, 1981), respectively. Available phosphorus and total potassium were estimated by Bray and Krutz method (Bray and Krutz, 1945) and by flame emission technique respectively. Exchangeable calcium and magnesium were estimated by standard methods (Jackson, 1973). Heavy metals were also determined using standard protocols (Lindsay and Norvel, 1978). All the determinations were carried out in triplicates.

Microbial analysis

Samples of both polluted and non-polluted (control) soil were taken from different sampling sites 0.1 g of soil were serially diluted in 90 mL ringer solution up to 10^{-6} dilutions and 1 mL aliquot was pour-plated in different selective media (Table 1) purchased from SRL, New Mumbai. The plates were incubated at 25 ± 10 °C. All the tests were done in triplicates. The special microorganism like mineralizers and polymer degraders are enumerated using the specific media following standard microbiological methods (Swiontek Brzezinska *et al.*, 2014; Aaisha and Barate, 2016). After the incubation period, the microbial colonies were counted as colony forming units (CFU/g) of the soil sample. The colony characteristics were observed, and single colonies were isolated and sub cultured on the respective media and the representative single colonies were identified using Bergey's manual of determinative bacteriology (Holt et al, 1994). Identification of yeast and fungi were done as per the available standard manual (Robinson, 2011).

Table 1: List of selective media used for the isolation and cultivation of soil microorganism.

Culture media used	Microorganism grown
Nutrient agar	Bacteria
Cooke rose bengal agar	Fungi
Actinomycetes isolation agar	Actinomycetes
Malt extract agar	Yeast
Jensen's media	Phosphate solubilizing soil microorganism
Starch agar media	Starch hydrolyzing microorganism
King's media	*Pseudomonas*

Statistical analysis

Statistical analysis of all the parameters were carried out through one-way ANOVA (SPSS Version 20). Conclusions were made using the arithmetic mean of the triplicate. *p*-value of ≤ 0.05 was found to be significant.

RESULTS

The physical properties of the soil like percentage of silt, sand and clay particle were not significantly altered even after pollution with the coffee processing wastes (*p*-value 0.66, 1.00, 0.77 respectively) (Figure 1). The polluted soil was found to be acidic (Figure 2a) and one soil sample taken from Somvarpet taluk showed a pH of 4.76. The E.C was significantly increased in polluted soil (Figure 2c.) The O.C of the polluted soil was high compared to the normal soil. The mean value was found to be 4.66 % (Figure 2b). The concentration of the macronutrients such

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

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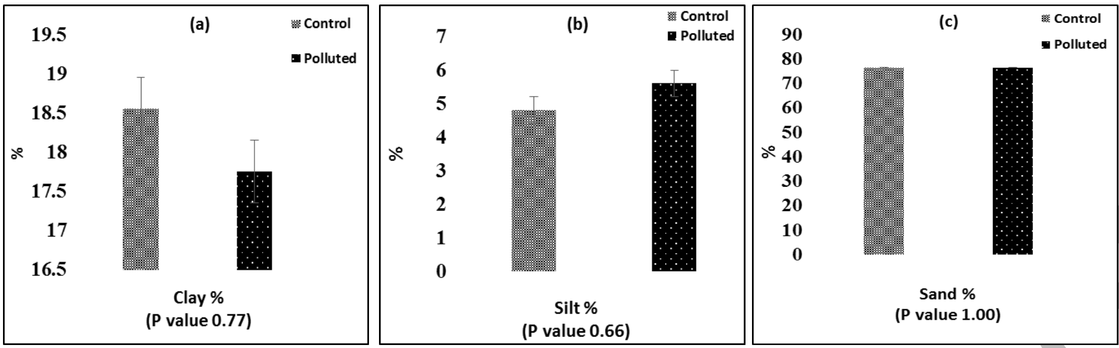

Figure 1: Analysis of physical properties of polluted and control soil (a) clay, (b) silt, (c) sand.

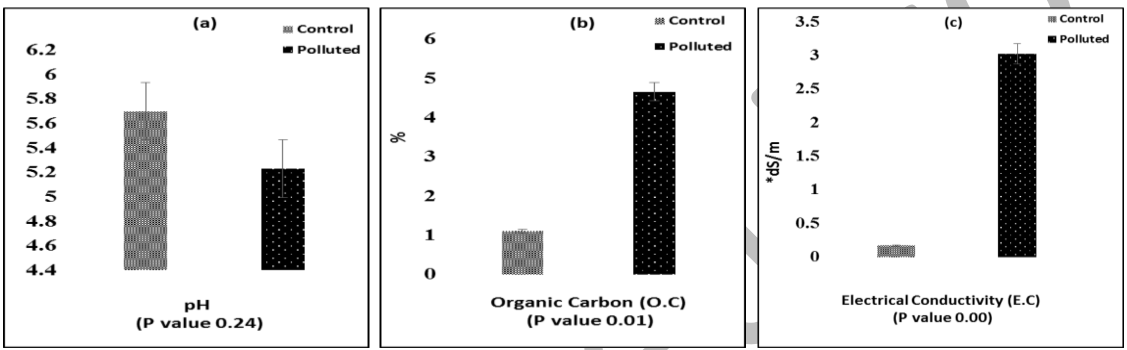

Figure 2: Analysis of physio-chemical properties of polluted and control soil (a) pH, (b) organic carbon content, (c) electrical conductivity.

as nitrogen (N), phosphorus (P), potassium (K) were found to be higher in polluted soil, out of which potassium content (11,088 kg/ha) was very significant (Figure 3a). The secondary nutrients such as calcium (Ca), magnesium (Mg) and sulphur (S) concentrations were high in soil polluted with coffee processing wastes (Figure 3b). Even the concentration of micronutrients such as boron (B), iron (Fe), nickel (Ni) were found to be high in polluted soil (Figure 3c). The concentration of phytotoxic chemicals such as caffeine, polyphenols and tannins in the polluted soil was highly significant (*p*-value of 0.00) (Figure 4). The caffeine content of the polluted soil was found to be 1.19 mg/g of soil. The average concentration of the chlorogenic acid in the polluted soil was found to be 0.600 mg/g of soil and the tannin content was 0.48 mg/g. The concentration of these phytotoxic chemicals in the corresponding control soil was negligible. The fungal population was more in polluted soil (Figure 5a). The colony forming units of plant growth promoting microorganism like starch hydrolyzing microorganism, pectinolytic microorganism, chitinolytic microorganism, nitrogen fixing microorganism and *Pseudomonas* were declined in soil polluted with coffee processing wastes (Figure 5b).

DISCUSSION

The soil pH is a very important factor of soil fertility. The soil polluted with the coffee processing wastes was found to be acidic compared to the control soil, thus demanding more liming agents. Liming often causes a temporary flush of soil microbial activity (Haynes and Naidu, 1998). The physical characteristics of the soil like percentage of sand and clay particles were not get altered even after pollution with coffee processing waste. The polluted soil was rich in organic carbon compared to the normal soil which indicates that the coffee processing waste contain high concentration of organic matter. The physical, chemical and biological properties of the soil get enhanced with the increased organic content of the soil. The concentration of primary nutrients, secondary nutrients and micronutrients were significantly high in polluted soil. The increased nutrient status of the polluted soil along with the high organic carbon content can be exploited by the coffee planters if the coffee processing wastes are scientifically processed.

The elevated concentration of phytotoxic chemicals such as caffeine, polyphenols and tannins in the polluted soil are detrimental. Caffeine, chemically 1,3,7-trimethyl xanthine, is a natural product from several plants. Caffeine in lower doses has stimulatory effect on growth and yield in plants while the higher doses have inhibitory

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

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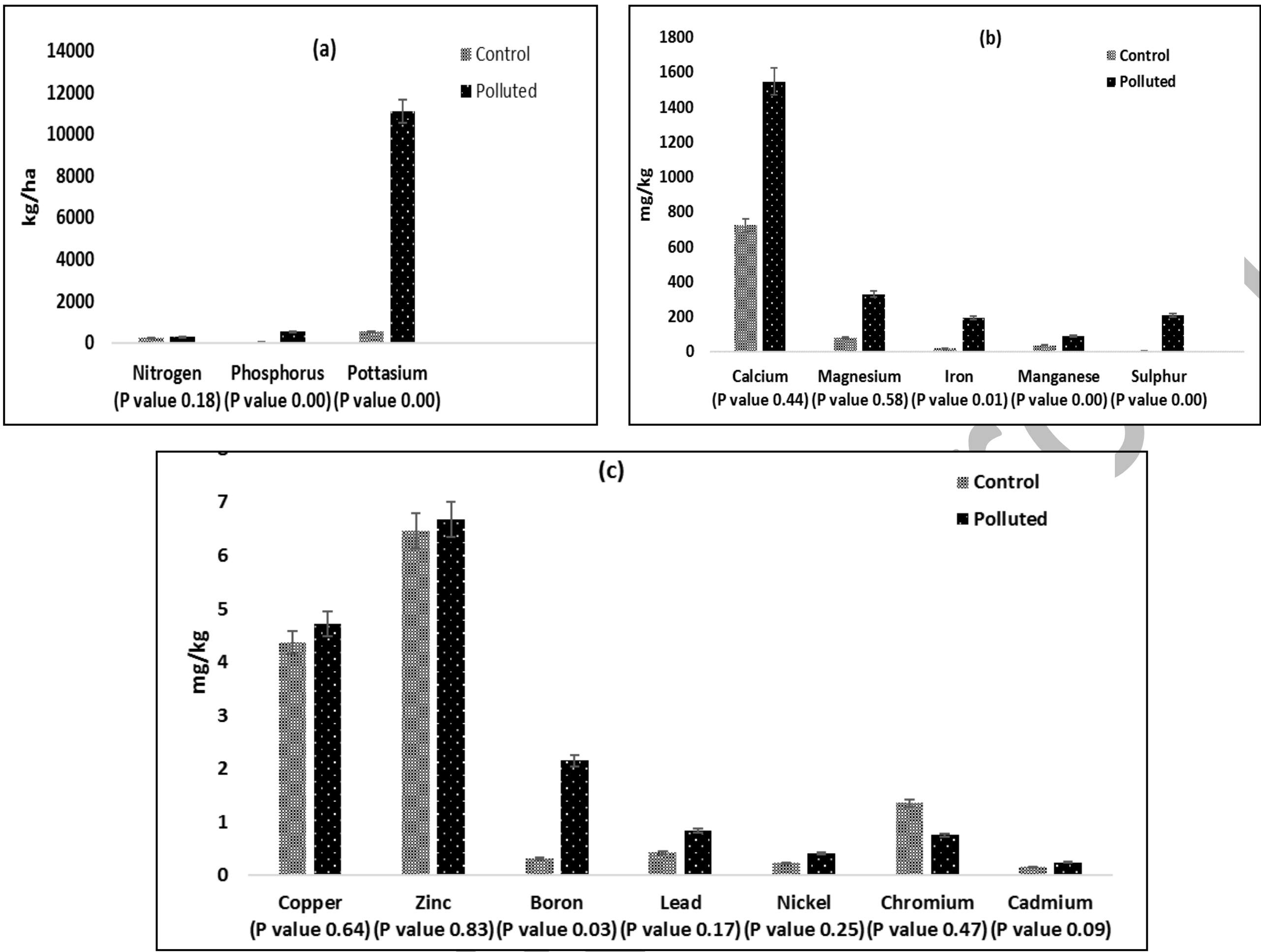

Figure 3: Analysis of plant nutrients in the polluted and control soil (a) macro nutrients, (b) micro nutrients, (c) other elements.

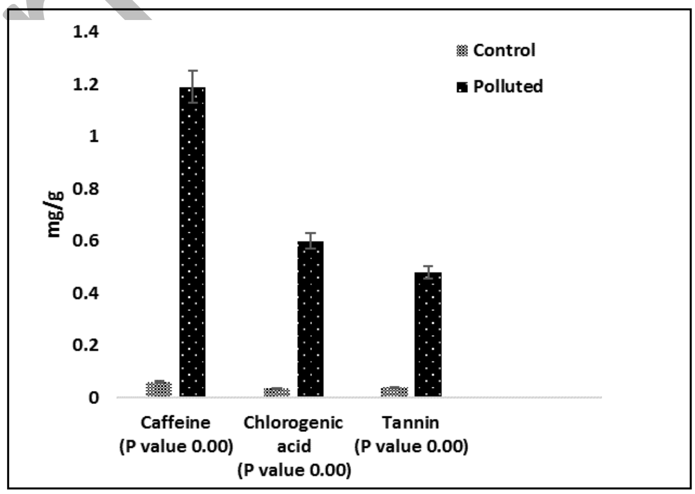

Figure 4: Analysis of phytotoxic chemicals in the polluted and control soil.

ISSN (print): 1823-8262, ISSN (online): 2231-7538

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effect. *Arabidopsis* seedling showed growth retardation and early senescence when exposed to caffeine (Mohanpuria and Yadav, 2009). Chlorogenic acid is a family of esters formed between certain hydroxy cinnamic acid and quinic acid. These form the main component of the phenolic fraction of the coffee skin. Chlorogenic acids like caffeoyl quinic acids, feruloyl quinic acids and di caffeoyl quinic acids were identified in pulp. The chlorogenic acid-protein complex present in the coffee pulp can inhibit the enzyme indole acetic acid oxidase (Farah and Donangelo, 2006). Chlorogenic acid showed inhibitory effect on the germination of *Artemisia herba-alba* seedlings (Al-Charchafchi and Al-Quadan, 2010). The soil polluted with coffee waste contained 0.600 mg/g of chlorogenic acid on an average, which may cause severe pollution problems. Tannins are known for their low biodegradability thus remaining in the environment for extended period and bioaccumulate in the food chain. Coffee processing waste when released to the environment, tannin leachate occurs. The studies on tannins leached from coffee industry waste is very limited (Janissen and Huynh, 2018). The present study revealed that the soil polluted with coffee processing waste contained 0.48 mg/g of tannin on an average. The concentration found to be critical and necessary steps are to be taken to remove tannin before disposal of the wastes as landfills.

The fungal population was more in polluted soil compared to the control. The plant growth promoting microorganism like phosphate solubilizer (Rodríguez and Fraga, 1999), *Pseudomonas*, *Azotobacter* sp., starch hydrolytic microorganism, nitrogen fixing microorganism (Hayat *et al*., 2010), pectinolytic and chitinolytic microorganism (Swiontek Brzezinska *et al*., 2014) were significantly decreased in the soil polluted with coffee processing wastes. Thus, continuous disposal of coffee processing wastes as landfill will certainly destroy the useful microorganism of the soil. The toxicity studies of caffeine, chlorogenic acid and tannins on plant growth promoting microorganism are going on in our laboratory.

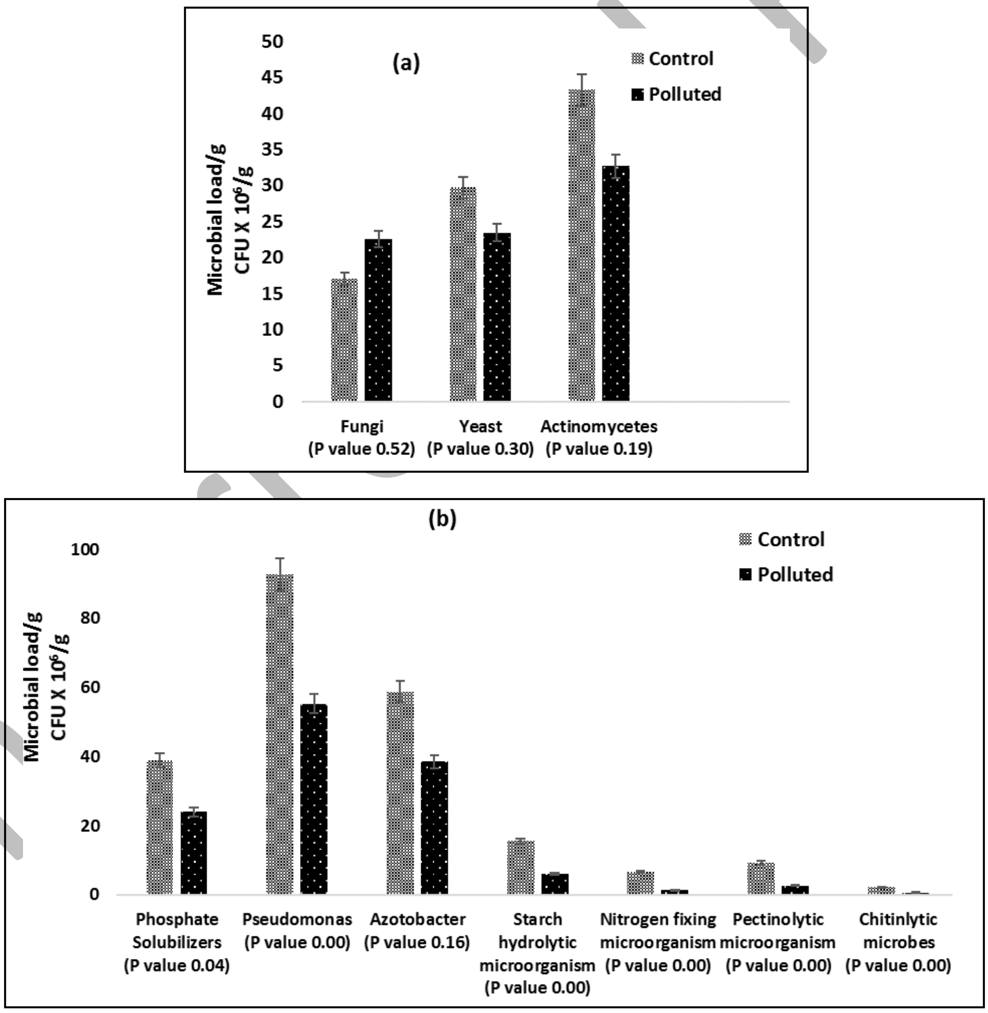

Figure 5: Microbial load of polluted and control soil (a) general microorganism, (b) plant growth promoting microorganism.

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

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CONCLUSION

The present work discussed the physio-chemical properties and microbial load of the soil polluted with coffee processing wastes. Coorg is referred as the coffee land of India and the main income for people of Coorg is coffee. The presence of toxic chemicals like caffeine, chlorogenic acid and tannins in the coffee processing wastes which were dumped as landfills in the Coorg, will pollute the soil and destroy the growth of useful microorganism. Thus, it can be concluded that the direct dumping of coffee processing wastes generates severe ecotoxicological problems.

ACKNOWLEDGEMENTS

Authors are thankful to Dr. S. Sandeep and Nisha, Kerala Forest Research Institute, Peechi, Thrissur, Kerala for the technical support given.

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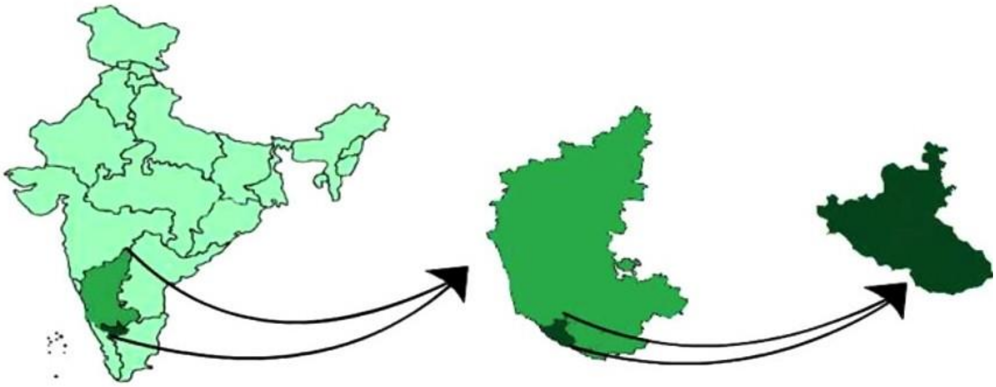
SUPPLEMENTARY INFORMATION

Supplementary Figure 1: Coffee processing waste as landfills in Coorg, Karnataka.



Supplementary Figure 2: Map of India showing Coorg district.

ISSN (print): 1823-8262, ISSN (online): 2231-7538

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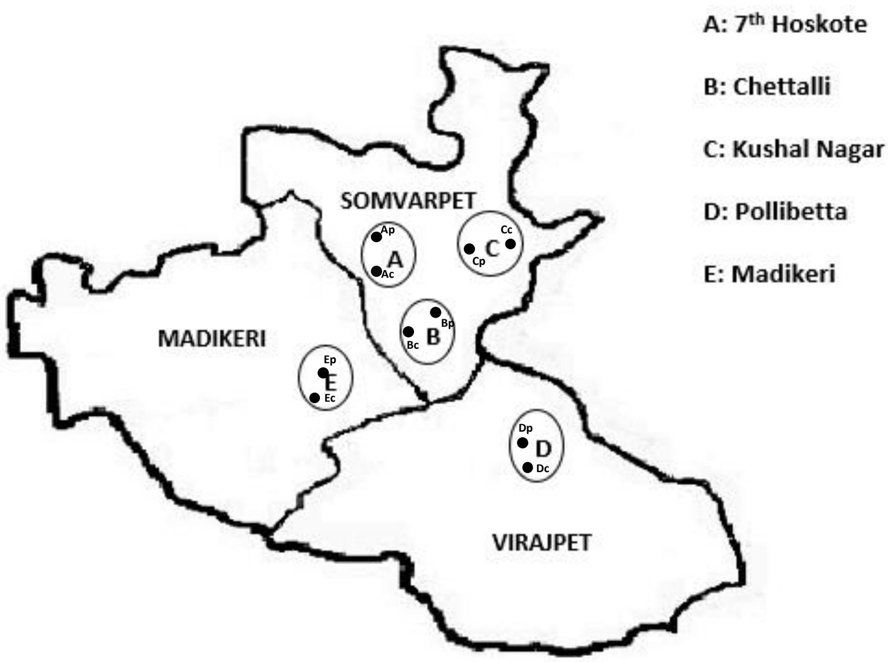

Supplementary Figure 3: Map of Coorg district showing the soil sampling sites.

Supplementary Table 1: The sampling spot with latitude and longitude.

State & district	Taluks	Places from which the soil samples collected and the sample code	Latitude and longitude
Karnataka, Coorg	Somvarpet	Chettalli Sample: Bc	12° 19' 37.58'' N 75° 49' 20.29'' E
		Sample: Bp	12° 27' 26.11'' N 75° 57' 36.65'' E
		7th Hoskote Sample: Ac	12° 26' 22.82'' N 75° 52' 26.82'' E
		Sample: Ap	12° 24' 54.25'' N 75° 44' 15.47'' E
		Kushal Nagar Sample: Cc	12° 27' 46.24'' N 75° 49' 20.29'' E
		Sample: Cp	12° 37' 33.65'' N 75° 48' 39.52'' E
	Madikeri	Madikeri Sample: Ec	12° 19' 37.58'' N 75° 57' 47.45'' E
		Sample: Ep	12° 22' 49.11'' N 75° 32' 57.38'' E
	Virajpet	Pollibetta Sample: Dc	12° 14' 28.63'' N 75° 54' 32.44'' E
		Sample: Dp	12° 15' 38.24'' N 75° 55' 11.50'' E

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