Screening of soil for assessment of toxicity of heavy metals to organisms

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Regular use of compost prepared from municipal solid waste is associated with the entry of heavy metals into the soil, which poses considerable risks to different components of the environment. Total metal content does not generally reflect the availability of metals for the expression of environmental risk because of rapid and strong interactions of the metals with different constituents of the soil. Hence, the present study was conducted to determine screening levels of Cd, Cr, Cu, Ni, Pb and Zn for a susceptible soil by following a widely recommended soil test procedure involving the extraction of these heavy metals with a dilute calcium chloride solution. Separate sets of pot-culture experiments were carried out for each of these heavy metals in graded dose levels (Cd at 0.02–20 mg kg^{-1}, Cr at 0.4–200 mg kg^{-1}, Cu at 1.6–800 mg kg^{-1}, Ni at 0.5–250 mg kg^{-1}, Pb at 0.4–150 mg kg^{-1} and Zn at 4.6–1000 mg kg^{-1}) added to an acidic, light-textured alluvial soil. Soil test screening levels were determined through three different approaches, namely, phytotoxicity, food contamination and soil microbial activity diminution. Except Pb, all other heavy metals significantly reduced the above-ground biomass growth of spinach. Activities of soil enzymes were adversely affected with increasing soil test values of the heavy metals. Screening levels of the heavy metals determined through food contamination and soil microbial activity diminution were much lower than those determined through phytotoxicity. The lowest values of these soil test screening levels of the heavy metals determined by three different approaches were considered to be protective for all target organisms and were found to be: 0.003 mg kg^{-1} Cd, 0.052 mg kg^{-1} Cr, 0.637 mg kg^{-1} Cu, 0.022 mg kg^{-1} Ni, 0.008 mg kg^{-1} Pb and 3.800 mg kg^{-1} Zn.

Keywords: Food contamination, heavy metals, microbial activity, screening, phytotoxicity, soil test.

WITH increasing urbanization–industrialization, heavy metals are increasingly entering agricultural ecosystems through waste products, and have become a focus of general interest for environmental protection. A significant part of about 70 million tonnes of municipal solid waste (MSW) generated every year in Indian cities is converted into compost for use in agricultural land for crop production. As MSW composts from most Indian cities contain considerable amounts of heavy metals[1], their prolonged use in crop production results in accumulation of these metals in the soil posing many risks to the health of both humans and the ecosystem. Risk to humans is expressed through contamination of the food chain, whereas environmental risks are expressed as phytotoxicity or ecotoxicity to soil flora and fauna.

While soil analysis for total heavy metal content may indicate their accumulation or contamination, it does not indicate whether the levels can pose any risk to the different components of the ecosystem. Toxicity of any metal in the soil essentially depends upon the readiness with which it is transferred to the targeted organisms[2]. With increasing evidence of trace metal pollution in all soils worldwide, there is a growing demand for methods to assess soil metal toxicity for the purpose of risk assessment and taking corrective measures. Soil testing methods have traditionally been developed and used for assessing the status of nutrients available to plants (i.e. soil fertility) for more than 40 years. However, there are several special considerations for the assessment of hazards due to heavy metal contamination, including the examination of effects on different components of the environment like humans, animals, plants and microorganisms[3].

Besides phytotoxicity, the presence of heavy metals in the soil affects the environment through food contamination because of the plants being grown in polluted soil and a decrease in soil microbial activity related to nutrient cycling processes[4–6]. Unlike soil tests for nutrient elements, there is a dearth of good data relating soil tests to environmental end-points for heavy metal toxicity. The current study was conducted to determine the screening levels of different heavy metals using a popular soil test procedure for a susceptible soil in which heavy metals are likely to cause toxicity at an early stage. A susceptible soil type has been defined here as that having low metal-fixing capacity and characterized by acidic chemical environment, and containing lower amounts of clay, organic matter and Fe oxides[2].

Bulk soil (Order Haplaquept) was collected from the surface layer (0–20 cm) of a cropland in the Cooch Behar District of West Bengal for the pot-culture experiments. These experiments were conducted in separate batches for each heavy metal (Zn, Cu, Pb, Cd, Ni and Cr) under open conditions. Metals were applied in graded dose levels to the soil (50% as salt and 50% through enriched compost): 4.6–1000 mg kg^{-1} Zn, 1.6–800 mg kg^{-1} Cu, 0.4–150 mg kg^{-1} Pb, 0.02–20 mg kg^{-1} Cd, 0.5–250 mg kg^{-1} Ni and 0.4–200 mg kg^{-1} Cr. Properties of the soil and MSW compost materials used in the experiment and detailed method for preparing experimental material are mentioned elsewhere[7]. Spinach (*Spinacia oleracea* L.), a widely grown leafy vegetable crop in India, has been used as the test crop for this study. It is known to have a high heavy metal uptake capacity so that concentration

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Table 1. Effect of soil test values of different heavy metals on their concentrations in leaf and yield of leaf biomass of spinach

Treatment	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
Cd										
STV	0.013	0.055	0.063	0.079	0.080	0.113	0.136	0.153	0.148	0.174
RY	100	98	86	89	76	79	73	70	53	50
Leaf-M	1.9	38.2	62.6	77.0	81.2	88.2	97.9	107.6	105.2	66.3
Cr										
STV	0.008	0.014	0.036	0.067	0.067	0.079	0.174	0.218	0.348	0.582
RY	100	105	107	102	105	110	107	104	97	56
Leaf-M	5.3	6.8	7.9	12.3	15.2	13.7	13.5	18.2	23.5	30.9
Cu										
STV	0.057	0.356	0.587	0.854	1.009	1.468	1.581	1.795	2.204	2.210
RY	100	100	100	103	91	95	66	27	15	9
Leaf-M	42.4	45.5	52.1	57.8	72.7	84.7	77.7	97.2	108.5	120.3
Ni										
STV	0.003	0.092	0.158	0.229	0.368	0.679	1.143	1.629	1.783	2.094
RY	100	108	98	95	94	95	90	68	68	66
Leaf-M	5.6	13.2	25.5	29.7	39.1	45.3	51.2	62.3	76.7	90.5
Pb										
STV	0.003	0.004	0.006	0.007	0.008	0.010	0.011	0.012	0.015	0.017
RY	100	106	118	116	109	107	108	101	83	93
Leaf-M	3.0	4.4	6.2	6.4	8.3	10.6	7.6	8.2	11.5	13.2
Zn										
STV	0.06	0.45	2.02	4.50	7.97	8.18	14.13	15.26	15.58	17.51
RY	100	102	105	99	108	99	85	70	66	59
Leaf-M	98	655	1081	1297	2022	2242	2252	2232	2752	2542

STV, Mean soil test value (mg kg^{-1}); RY, Mean relative (in respect to control treatment) biomass yield (%); Leaf-M, Mean metal concentration in spinach leaf (μg g^{-1} dm).

limits determined with this crop could also be considered protective for other crops with lower heavy metal uptake capacity[8].

To estimate soil test level of metals, suspension of 10 g of air-dried soil and 25 ml of 0.01 M $CaCl_2$ solution was shaken for 4 h on a reciprocal shaker at 200 rpm at 25°C, followed by filtration through Whatman No. 42 filter paper. Metals in all the soil and plant extracts (with hot HNO_3 and $HClO_4$ acid) were analysed by inductively coupled plasma emission spectroscopy (PerkinElmer make, Optima 2100DV).

Sub-samples of soil collected immediately after the harvest of the spinach biomass were analysed for soil enzyme activity using four different substrates: 2,3,5-triphenyl tetrazolium chloride (TTC, for measuring dehydrogenase activity), fluorescein diacetate (FDA, for measuring proteases, lipases and esterases activities), *p*-nitrophenyl phosphate (PNPP, for measuring acid phosphatase activity) and *p*-nitrophenyl sulphate (PNPS, for measuring arylsulphatase activity). Procedures for measurement of the above soil enzyme activities are mentioned elsewhere[7]. Analysis of variance and least significance differences for analysed parameters were carried out with statistical software SPSS ver. 9.0 for each parameter.

The above-ground biomass of spinach was significantly reduced at $CaCl_2$-extractable levels (or soil test levels) of and higher than 0.080 mg kg^{-1} Cd, 0.582 mg kg^{-1} Cr, 1.581 mg kg^{-1} Cu, 1.63 mg kg^{-1} Ni and 15.258 mg kg^{-1} Zn (Table 1). Doses of Pb did not have any significant effect on the above-ground biomass of spinach. From the best-fit equations for data-pair between available metals (*x*-axis) and relative biomass yield (*y*-axis), the soil test levels corresponding to 80% relative yields were found to be 0.100 mg kg^{-1} Cd, 0.465 mg kg^{-1} Cr, 1.357 mg kg^{-1} Cu, 1.350 mg kg^{-1} Ni and 13.987 mg kg^{-1} Zn. We considered 20% growth retardation (PT_{20}) as a better indicator for phytotoxicity of heavy metals for the purpose of determining their ecologically limiting concentrations in the soil, as 50% growth retardation is considered too severe a loss for farmers.

Results of concentration of metals in spinach leaf in response to their soil test metal levels are presented in Table 1. In the range of soil test levels, concentration of Cd and Pb in the aboveground biomass tissue followed a linear relationship with their extractable levels in the soil. However, data for the above-ground biomass Cr, Ni and Zn levels fit best to power functions ($R^2 = 0.937$**, 0.953** and 0.951** respectively), indicating decreasing

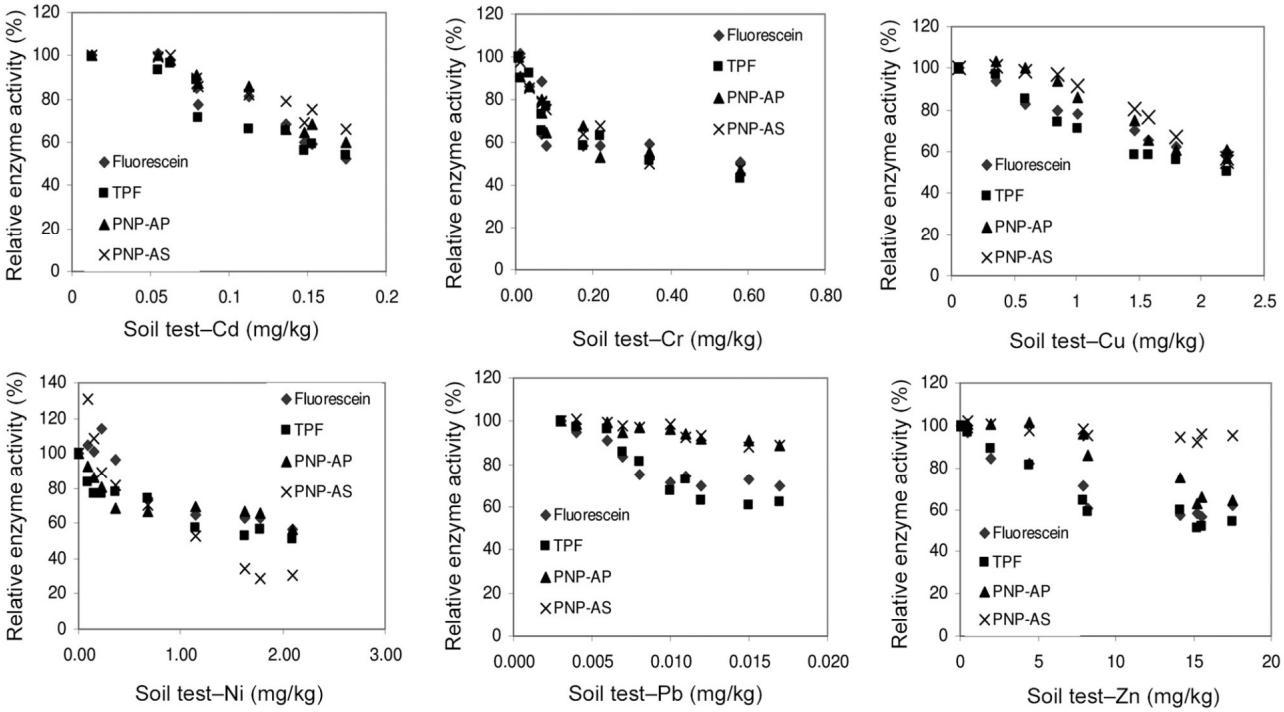

Figure 1. Effects of $CaCl_2$-extractable metals in soil on relative soil enzyme activities (fractions of control soil enzyme activities). Fluorescein – Activities of esterase + lipase + protease; TPF – Dehydrogenase activity; PNP-AP – Acid phosphatase activity; PNP-AS – Arylsulphatase activity.

metal transfer at higher soil test metal levels. Screening level of a metal based on food (in this case leaf) contamination has been considered to be the maximal soil test value that does not increase its concentration in the edible food crop above the background level (i.e. concentration range found in the crop grown in uncontaminated soil). The method followed for determination of screening levels based on this approach has been elsewhere[7]. In brief, maximal value of the range [mean ± 3* (standard deviation)] for metal concentration in spinach biomass after control (metal-free) treatment (24 replications) was taken as the upper limit of background metal concentration (C_{ul}). From the best-fit equations for lines obtained by plotting biomass metal concentrations versus soil test-metal concentrations, soil test values corresponding to C_{ul} were considered as screening levels based on food contamination. These values were computed as 0.003 mg kg^{-1} Cd, 0.176 mg kg^{-1} Cr, 0.637 mg kg^{-1} Cu, 0.022 mg kg^{-1} Ni, 0.008 mg kg^{-1} Pb and 0.068 mg kg^{-1} Zn. However, Zn is also considered to be important for human nutrition and its deficiency is a critical health problem affecting about one-third of the world's population[9]. Hence, contamination of edible parts of plants with Zn can even be considered desirable, which implies that the food chain contamination approach may not be the most suitable method to determine the maximal concentration limit for this element.

Nutrient cycling in the soil involves biochemical, chemical and physico-chemical reactions, with biochemical processes being catalysed by enzymes released by microorganisms[10]. Assays of enzyme activities are considered to be important in assessing the impact of metal pollution on the soil environment[11]. Effects of soil test metal levels on relative enzyme activities are presented in Figure 1, wherein the measure of any enzyme activity after a particular type of treatment is expressed as the proportion of the product concentration of the enzymatic reaction in relation to the product concentration in the control treatment. Some or all of the biochemical processes involving different substrates have been found to be adversely and significantly affected by the soluble, exchangeable fraction of all the metals. Dehydrogenase activity as indicated by the magnitude of reduction of TTC was significantly decreased with soil test values as follows: 0.080 mg Cd kg^{-1}, 0.174 mg Cr kg^{-1}, 0.854 mg Cu kg^{-1}, 1.14 mg Ni kg^{-1}, 0.012 mg Pb kg^{-1} and 4.50 mg Zn kg^{-1} or more. Hydrolysis of FDA was significantly reduced with soil test levels of 0.080 mg Cd kg^{-1}, 0.174 mg Cr kg^{-1}, 0.587 mg Cu kg^{-1}, 1.63 mg Ni kg^{-1}, 0.011 mg Pb kg^{-1} and 4.50 mg Zn kg^{-1} or more. Hydrolysis of phosphate from *p*-nitrophenyl phosphate (indicative of phosphatase activity) was decreased significantly with soil test levels of 0.174 mg Cd kg^{-1}, 0.067 mg Cr kg^{-1}, 1.47 mg Cu kg^{-1}, 0.229 mg Ni kg^{-1} and 14.13 mg Zn kg^{-1} or more. In contrast, hydrolysis of sulphate from *p*-nitrophenyl sulphate (indicative of arylsulphatase activity) was decreased significantly only with soil test levels of 0.067 mg Cr kg^{-1} and 0.679 mg Ni kg^{-1} or more.

Table 2. Best-fit equations for lines obtained by plotting biochemical activities versus $CaCl_2$ extractable metal levels in soil

Dependent variable (y)[a]	Regression equations	R^2	ED_{20} (mg kg^{-1} soil)
Independent variable (x): soil test–Zn (mg kg^{-1})			
Fluorescein	$y = 0.111x^2 - 3.1951x + 55.124$	0.9627	3.88
TPF	$y = 0.0512x^2 - 1.5735x + 25.818$	0.974	3.8
PNP-AP	$y = 0.024x^3 - 0.7829x^2 + 3.2664x + 145.4$	0.966	11.34
Independent variable (x): soil test–Cu (mg kg^{-1})			
Fluorescein	$y = -8.8861x + 47.107$	0.9494	0.95
TPF	$y = 2.0125x^2 - 11.043x + 27.745$	0.9796	0.7
PNP-AP	$y = 22.247x^3 - 79.947x^2 + 42.642x + 140.48$	0.9724	1.22
Independent variable (x): soil test–Cd (mg kg^{-1})			
Fluorescein	$y = -539.44x^2 - 65.995x + 54.014$	0.9213	0.104
TPF	$y = 5665.6x^3 - 1558x^2 + 38.97x + 23.811$	0.9101	0.087
PNP-AP	$y = 29942x^3 - 9928.8x^2 + 514.44x + 141.8$	0.9549	0.111
Independent variable (x): soil test–Pb (mg kg^{-1})			
Fluorescein	$y = -1E + 06x^3 + 164041x^2 - 4050.3x + 65.183$	0.9436	0.0078
TPF	$y = 1E + 07x^3 - 298166x^2 + 1103.6x + 27.672$	0.9655	0.0078
Independent variable (x): soil test–Ni (mg kg^{-1})			
Fluorescein	$y = -25.779x^4 + 111.07x^3 - 142.3x^2 + 34.347x + 61.556$	0.9561	0.678
TPF	$y = 8.6874x^2 - 34.177x + 81.161$	0.8934	0.283
PNP-AP	$y = -23.794x^3 + 84.32x^2 - 90.518x + 86.439$	0.9724	0.239
PNP-AS	$y = 23.694x^2 - 106.03x + 157.48$	0.9198	0.509
Independent variable (x): soil test–Cr (mg kg^{-1})			
Fluorescein	$y = -785.63x^3 + 776.19x^2 - 221.52x + 40.388$	0.842	0.052
TPF	$y = -889.26x^3 + 918.33x^2 - 306.92x + 75.433$	0.9064	0.054
PNP-AP	$y = -1229.1x^3 + 1282x^2 - 421.76x + 101.13$	0.9255	0.054
PNP-AS	$y = -947.21x^3 + 1134x^2 - 471.11x + 147.87$	0.9544	0.071

[a]Fluorescein – µg Fluorescein/g soil/h; TPF – µg 1,3,5-triphenyl formazan/g soil/24 h; PNP-AP (in acid phosphatase measurement) – µg *p*-nitrophenol/g soil/h; PNP-AS (in arylsulphatase measurement) – µg *p*-nitrophenol/g soil/h.

The concept of an ecological dose (ED_{50}) was developed to facilitate easy quantification of the effects of pollutants on microbe-mediated biochemical processes of ecological significance in various ecosystems. It is defined as the toxicant concentration that inhibits a microbe-mediated ecological process by 50% (ref. 12). However, a 50% reduction in a basic ecological process may be extreme for the continued functioning of agricultural soil. The lower percentage of inhibition (20%, ED_{20}) is considered to be a more suitable criterion to protect soil quality in a soil ecosystem subjected to heavy metal pollution[13,14]. The ED_{20} value in any enzymatic reaction is derived from the equations for best-fit lines plotted for enzymatic activity versus soil metal concentrations, and predicts the soil metal level corresponding to 20% inhibition of enzyme activity (Table 2).

The ED_{20} levels of Cd, Cu, Cr, Pb and Zn were generally low when using the TTC and FDA method of biochemical analysis, indicating maximal toxicity of these metals towards dehydrogenase, proteases, lipases and esterases. However, Ni and Cr showed high toxicity towards acid phosphatase, as indicated by their low ED_{20} levels determined using *p*-nitrophenyl phosphate (PNPP). In general, heavy metals showed relatively lower toxicity towards arylsulphatase activity. The lowest of all the ED_{20} values for any metal determined through different biochemical approaches is taken as the maximal concentration of that metal which might not show any adverse effects on soil microbial activity. These values were 0.087 mg kg^{-1} for Cd, 0.052 mg kg^{-1} for Cr, 0.700 mg kg^{-1} for Cu, 0.239 mg kg^{-1} for Ni, 0.008 mg kg^{-1} for Pb and 3.8 mg kg^{-1} for Zn.

As the lowest among these maximal screening levels of soil test values determined using the above three different approaches can protect all target organisms, the values of 0.003 mg kg^{-1} Cd, 0.052 mg kg^{-1} Cr, 0.637 mg kg^{-1} Cu, 0.022 mg kg^{-1} Ni, 0.008 mg kg^{-1} Pb and 3.800 mg kg^{-1} Zn can be considered to be the screening levels for assessing heavy metals toxicity in MSW compost-amended soil. In the field situation, contaminated organic amendments are normally confined to the plough layer (the contaminated unfavourable zone), although the plant roots may proliferate in a deeper layer (the uncontaminated favourable zone). This is likely to dilute metal concentration in the plant tissues and be associated with lower metal transfer coefficients. Experiments conducted in

small containers (such as the pot-culture experiments described in this study) allow intensive root contact with the contaminated soil matrix. Hence, plant tissue concentrations measured in these pot-culture experiments would be higher than what would be seen in actual field situations. Therefore, screening levels reported and recommended by this study are likely to be on the lower side and hence can be assumed to be safer for target organisms under actual field situations. Due to high heavy metal uptake capacity of the test crop, i.e. spinach, determined soil test screening levels would also be protective for other crops with lower uptake efficiency. The order of screening levels of soil test values was Zn > Cu = Ni > Cr > Cd based on phytotoxicity, Zn > Cu > Ni > Cd > Cr > Pb based on adverse effects on soil microbial activity and Cu > Cr > Zn > Ni > Pb > Cd based on food contamination. The similarity in the order of the screening levels of soil test values for the former two approaches indicates that heavy metals show similar patterns of toxicity to both plant and soil microbes. Screening levels of soil test values determined for all the heavy metals by the phytotoxicity approach were considerably higher than the screening levels determined by the microbial activity diminution and food chain contamination approaches. This indicates that adverse effects on microbial activity and contamination of the food chain by heavy metals occurred much earlier than their adverse effects on plant growth.

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