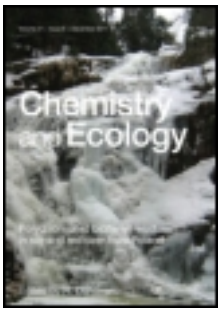

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Jun-Hui Zhang[a] & Wei-Wei Fan[a]
[a] School of Life Science, Taizhou University, Taizhou, People's Republic of China
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Metal partitioning and relationships to soil microbial properties of submerged paddy soil contaminated by electronic waste recycling

Jun-Hui Zhang* and Wei-Wei Fan

School of Life Science, Taizhou University, Taizhou, People's Republic of China

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The interactions between microbial properties and metal partitioning were investigated in paddy soils during the cultivation of rice in field experiments. Samples from an electronic waste-recycling centre (e-waste centre) were used as the contaminated sample. Multiple regressions analysis showed that activity of soil enzymes and the abundance of microbial physiological groups were affected by residual Cd, residual Zn, exchangeable Cu, exchangeable Zn and Fe–Mn-oxidising Cd. The residual Cd concentration could predict 66.7, 35.5 and 62.4% of the variance in the activity of sulfate reductase, peroxidase and urease, respectively, while the concentrations of exchangeable Cu, exchangeable Zn and Fe–Mn-oxidising Cd explained 43.2, 9.9 and 65.2% of the variance in catalase, sulfate reductase and acid phosphatase, respectively. The contents of residual Cd and residual Zn could predict 89.0 and 42.7%, respectively, of the variations in the abundance of total bacteria and metal-resistant bacteria. However, metal partitioning was affected by soil microorganisms. Organic Zn and Fe–Mn-oxidising Cd were easily affected by soil bacteria and sulfur-oxidising bacteria. The abundance of soil bacteria explained 69.8 and 64.7% of the variance in organic Zn and Fe–Mn-oxidising Cd, and sulfur-oxidising bacteria explained 21.0% of the variance in Fe–Mn-oxidising Cd.

Keywords: e-wastes; metal partitioning; paddy soil; soil enzyme; soil microorganism

1. Introduction

Improper electronic waste (e-waste) processing provides many routes through which toxic substances can pollute the environment.[1] The accumulation of heavy metals, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) in soil, sediment,[2,3] air [4] and food [5,6] as a result of long-term improper e-waste recycling has been reported in many areas. The presence of multiple contaminants may present substantial challenges to the maintenance of a phylogenetically and functionally diverse soil microbial community. Thus, the presence of pollutants can lead to changes in the soil community structure.[7,8] However, the effect of improper e-waste recycling on soil enzymes and microorganisms has been rarely studied, especially under field conditions.

It has been proposed that soil enzymes and microorganisms are valuable indicators of the ability of soil to function and recover after being disturbed (resistance and resilience). Although a single pollutant can reduce soil enzyme activity [9,10] or change the soil community structure,[11] the toxicity of pollutant mixtures, particularly in relation to microbial activity, has received much less attention. In addition, most reported studies focus on the total elemental content of soils,

*Corresponding author. Email: zhangjunhui@tzc.edu.cn

with little or no consideration for chemical partitioning and bioavailability. However, a consensus exists concerning the importance of bioavailability and metal partitioning in trace metal toxicity studies. Here, we focus on the toxicity and bioavailability of a mixture of metals in the submerged paddy soil surrounding an e-waste recycling area.

Soil microorganisms can influence the mobility and bioavailability of a metal through a variety of processes.[12] These include not only indirect changes in the physical and chemical properties of the soil, but also direct enzymatic action on these compounds, which serve as terminal electron acceptors, and the synthesis of metabolites with chelate ability.[13] Thus, the activity of soil microorganisms may have an appreciable effect on the metal fractionation/partitioning pattern. However, there are few reports currently available in the published literature on this subject. In this study, two paddy soils contaminated by e-waste processing and one control soil were studied. Metal partitioning, soil enzyme activities and the abundance of microbial physiological groups were continually documented during cultivation of the rice crop. A further aim was to establish empirical relationships between metal partitioning in the soils and their microbial properties using multiple linear regression analysis. These relationships were then used to investigate the interaction of the microbial properties and the metal partitioning.

2. Materials and methods

2.1. *Research area*

The research was conducted at an old e-wastes centre (28°29′ N, 121°20′ E) located in the southeast of Zhejiang province, China (Figure 1). This area had been used for e-waste processing for over 20 years. The region has a northern sub-tropical monsoon climate. The mean annual precipitation is 1600–1700 mm, of which 60.2% is received between May and September. The annual mean air temperature is 17°C, with a maximum of 40.8°C, and a minimum of −9.9°C.

2.2. *Experimental set-up*

In July 2006, two polluted sites (G1 and G2) in the e-wastes processing centre and one control site (CK) were chosen for study (Figure 1), each site included three randomised replicate plots of 5 m × 4 m. G1 was located near a wastewater outfall from an e-waste processing factory. G2 was located near a dumping site at which a large amount of unsalvageable materials was piled. CK

Figure 1. Map showing the study area. The two polluted sites were located in an e-waste recycling centre. CK is a clean paddy soil. The map of Taizhou city is from Ledin [45].

was located 120 km from the recycling centre. Paddy rice seedlings (*Oryza sativa* L, XIEYOU 9308) were transplanted in rows 20 cm apart. Each hill contained three seedlings (21 days old), which were hand dibbled at a spacing of 15 cm. All plots were under simulated flood conditions for 105 days, and were drained approximately a week before harvest. Plant nutrients were added three times (at the seedlings stage, prior to transplanting and at florescence) in the form of urea ($50\,kg\,ha^{-1}$). Bulk soil samples were collected at 0, 15, 20, 50 and 80 days after transplanting. The five sampling times were corresponded to stages prior to transplanting, tiller stage, mid tassel stage, florescence and maturity stage of the rice development, respectively. For each sampling, five subsamples per plot were randomly collected at a depth of 15 cm with a hand-driven shovel and then mixed to constitute a single sample per plot. The samples were sieved to < 2 mm and stored in clean polyethylene bags at 4°C until analysis.

Chemical analyses were performed on dry soil samples. Pseudo-total metal concentrations in the soil samples were determined after digestion with a combination of concentrated HNO_3 and $HClO_4$. The Cu, Zn and Cd in the soil samples were sequentially extracted, following the method of Tessier,[14] into five phases, and were operationally defined as the exchangeable, carbonate, Fe–Mn-oxidising, organic and residual fractions. The metal contents of all the solutions were determined by inductively coupled plasma atomic emission spectrometry (ICP-OES, Optima 2100 DV, Perkin–Elmer, USA). The recovery rates calculated from the sum of the five fractions over the total trace metal concentrations, determined using 'pseudo' total digestion, ranged between 75 and 110%. The soil pH was measured using a pH meter with a 1 : 2.5 soil/water suspension.[15] The soil organic matter was determined by the wet combustion method of Önorm.[16] The total soil nitrogen (TN), total soil phosphorous (TP) and cation-exchange capacity (CEC) of soil samples were determined according to Bremner and Mulvaney.[17] Clay was determined according to R. Öhlinger.[18]

The soil enzyme activities and the abundance of soil microorganisms were measured using fresh wet samples. Catalase was analysed using potassium permanganate titration.[19] Peroxidase was analysed using pyrogallol as a substrate.[20] The urease activities in the soil samples were measured as the capacity to catalyse the transformation of urea into ammonium.[21] Sulfate reductase activity was determined by the EDTA titration method.[22] Invertase was analysed using sucrose as the substrate.[23] Acid phosphatase was determined by the release and detection of *p*-nitrophenol (PNP), using 0.1 M phenyl-phosphate disodium as a substrate.[24] The abundances of ammonia oxidiser, denitrifier, sulfate-reducing bacteria (SRB) and sulfur-oxidising bacteria (SOB) were determined by the most probable number (MPN) technique. The abundance of total aerobic or facultative anaerobe bacteria (total bacteria) and metal-resistant bacteria were accounted by the pour plate method. The former were evaluated by exposing the soil microbial communities to a defined mineral medium,[25] while the latter were evaluated by exposing the above medium with 2 mM $Pb(NO_3)_2$ supplied. All values were reported on an oven dry basis.

All experimental data are reported as the mean ± standard deviations. The data were analysed by one-way analysis of variance (ANOVA) and multiple stepwise regressions, using Statistical Package for the Social Sciences (SPSS) 11.5 for Windows. Multiple comparisons were statistically evaluated by the ANOVA and Tukey's test. The least significant differences (LSD) among mean values were calculated at $p < 0.05$.

3. Results

3.1. *Soil physicochemical properties*

The paddy soils differed greatly in their pH and trace metal contents (Table 1). Soils G1 and G2 were acidic, whereas a nearly neutral pH was found for the control soil. The total organic matter,

Table 1. Physicochemical properties and metal concentrations of the paddy soil samples taken from the e-waste contaminated soils and the control (mean ± SE).

	G1	G2	CK	Environmental quality standard for soils (China, Grade II, pH < 6.5)
pH	4.49 ± 0.08	4.40 ± 0.23	6.08 ± 0.04	
Organic matter ($g\,kg^{-1}$)	58.43 ± 4.72	44.30 ± 4.07	57.67 ± 3.58	
Total N ($mg\,kg^{-1}$)	2,230.00 ± 137.00	2,730.00 ± 179.00	2,390.00 ± 157.70	
Total P ($mg\,kg^{-1}$)	332.81 ± 17.71	419.68 ± 20.19	425.00 ± 18.74	
CEC ($cmol\,100\,g^{-1}$)	3.703 ± 0.44	5.477 ± 0.47	5.600 ± 0.23	
Clay (%)	7.62 ± 0.98	8.40 ± 1.18	8.12 ± 0.79	
Cd ($mg\,kg^{-1}$)	6.39 ± 0.30	16.04 ± 0.68	0.15 ± 0.03	0.3
Co ($mg\,kg^{-1}$)	63.34 ± 1.38	76.08 ± 1.87	8.72 ± 2.43	–
Cr ($mg\,kg^{-1}$)	18.58 ± 0.88	30.54 ± 0.69	6.33 ± 1.03	300
Cu ($mg\,kg^{-1}$)	298.64 ± 37.06	406.62 ± 40.21	32.08 ± 2.11	100
Fe ($mg\,kg^{-1}$)	33,882.00 ± 894.23	35,574.69 ± 907.18	15,598.68 ± 1046.56	–
Mn ($mg\,kg^{-1}$)	369.47 ± 30.12	345.39 ± 28.40	324.81 ± 24.66	–
Pb ($mg\,kg^{-1}$)	36.22 ± 2.17	46.70 ± 2.93	33.44 ± 1.33	250
Zn ($mg\,kg^{-1}$)	205.70 ± 40.88	255.75 ± 43.67	111.99 ± 37.77	200

TN, TP and clay content in all the three soil samples were approximately equivalent, whereas the CEC of soil G1 was 32.39 and 33.87% lower than those of G2 and CK, respectively, indicating that soil G1 had insufficient buffering capacity for metal contamination.

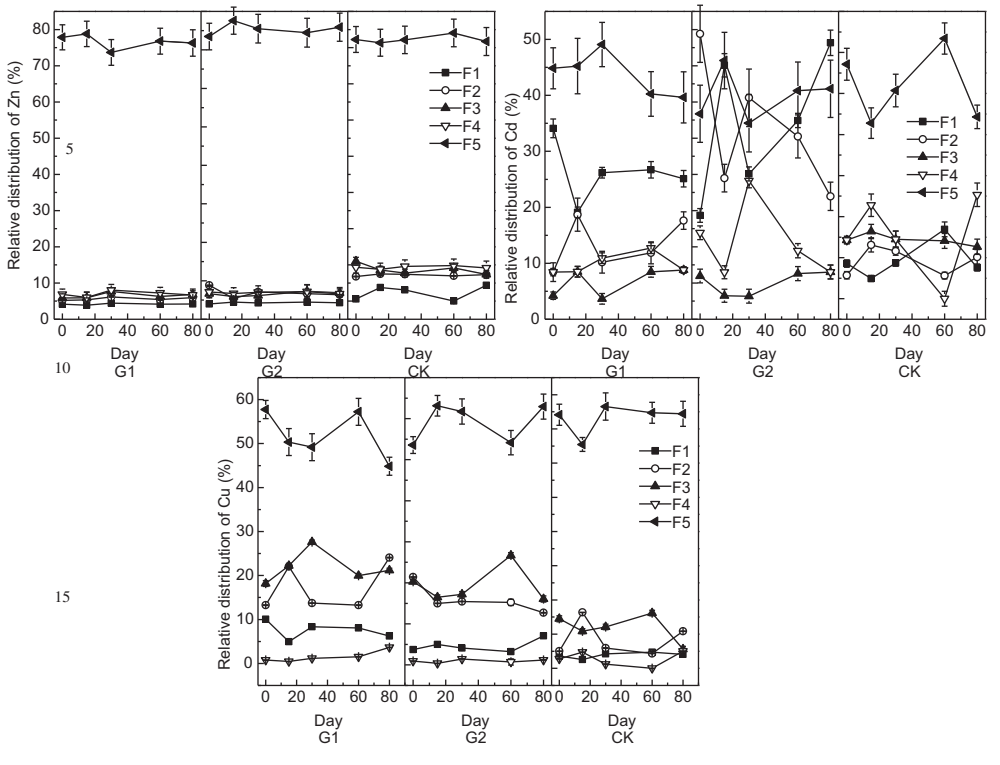

Figure 2. Chemical partitioning patterns for metals in the flooded paddy soil samples taken from the e-waste contaminated site and the control site. F1, exchangeable fraction; F2, carbonate fraction; F3, Fe–Mn-oxidising fraction; F4, organic fraction; F5, residual fraction.

The soils of sites G1 and G2 were significantly enriched with trace metals, with differences in Cd, Co, Cu, Cr, Fe and Zn concentrations between the two contaminated sites and the control site being the most noticeable (Table 1). The Cd, Co, Cu, Cr, Fe and Zn concentrations in site G1 were ~41.60, 6.26, 8.31, 1.93, 1.17 and 0.84 times higher than those in the control site. In addition, the Cd, Co, Cu, Cr, Fe and Zn concentrations in the G2 soil were ~106.90, 8.72, 11.67, 4.82, 2.28 and 2.28 times as the control soil. The Cd, Cu and Zn concentrations in the G1 and G2 soils exceeded the threshold of the Chinese environmental quality standard (GB 15618–1995, Grade II) [26] and therefore, have a potential to cause toxic ecological effects.

3.2. *Metal partitioning*

The partitioning patterns of Zn (Figure 2, expressed as the percentages of total content) were relatively constant over time. Most of the Zn at the three tested sites was only slightly available, as it was primarily bound to the residual fraction, ranging from 73.67 to 78.79%, 73.47 to 77.59% and 71.34 to 74.04% for soils G1, G2 and CK, respectively. However, the chemical distributions of Cu and Cd were variable during the rice development. However, the variations were not the same for metals in the different fractions. The majority of Cu in all the soil samples was associated with the residual fraction, which varied from 49.18 to 57.76%, 53.58 to 63.16% and 65.45 to 76.62% for soils G1, G2 and CK, respectively. Although Cu showed a high affinity for organic matter,[27] the organic fraction in the flooded paddy soil was very low, especially in G1 and G2. This may have contributed to the low pH of the polluted paddy soil. Cloutier-Hurteau once observed that the organic form of Cu was increased with the increasing of pH.[28] Although they varied over time,

Table 2. Data from multiple regression analysis between metal partitioning and soil characteristics ($n = 45$, 3 sites × 3 plots × 5 times).

Metal/fraction	Multiple linear regression	Statistics
$Zn_{exchangeable}$	$= 5.519 - 0.623\ Zn_{Fe-Mnoxidising} + 0.111 Cd_{organic} + 0.059\ Cd_{exchangeable}$	$R^2 = 0.812, p < 0.001$
$Zn_{carbonate}$	$= 99.945 - 0.999\ Zn_{residual} + 7.8 \times 10^{-5} Cd_{carbonate} - Zn_{exchangeable} - Zn_{carbonate} - 0.998\ Zn_{organic}$	$R^2 = 1.000, p < 0.001$
$Zn_{Fe-Mn\ oxidising}$	$= 0.667\, Zn_{organic} + 0.001\ SRB + 0.653\ pH - 1.627$	$R^2 = 0.795, p < 0.001$
$Zn_{organic}$	$= 20.524 + 8.8 \times 10^{-7}\ Bacteria_{total} + 0.057\ Cd_{residual} - 0.207\ Zn_{residual} - 0.002\ Denitrifier$	$R^2 = 0.976, p < 0.001$
$Zn_{residual}$	$= 87.085 - 2.805\ Zn_{organic} + 0.132\ Cd_{residual} + 0.063\ Cu_{residual}$	$R^2 = 0.895, p < 0.001$
$Cu_{exchangeable}$	$= 6.2 - 0.071\ P + 0.258\ Cd_{exchangeable} + 0.142\ Cu_{residual} + 1.645\ Zn_{carbonate} + 5 \times 10^{-5}\ Bacteria_{resist} + 0.062\ Cd_{organic}$	$R^2 = 0.990, p < 0.001$
$Cu_{carbonate}$	$= 51.883 - 3.587\ Zn_{organic} - 0.238\ Cd_{exchangeable} + 0.001\ Bacteria_{resist} + 0.002\ Ammonia\ oxidiser$	$R^2 = 0.895, p < 0.001$
$Cu_{Fe-Mn\ oxidising}$	$= 38.576 - 0.259\ Cu_{residual} - 0.338\ Cd_{organic}$	$R^2 = 0.684, p < 0.001$
$Cu_{organic}$	$= 0.168\ Cd_{organic} - 0.332$	$R^2 = 0.587, p = 0.001$
$Cu_{residual}$	$= 48.455 + 6.486\ Zn_{organic} - 0.529\ Cu_{carbonate} - 0.638\ Cd_{organic}$	$R^2 = 0.841, p < 0.001$
$Cd_{exchangeable}$	$= 48.313–0.928 Cd_{carbonate} - 3.988 Zn_{carbonate} + 0.001N - 0.331 Cd_{organic}$	$R^2 = 0.873, p < 0.001$
$Cd_{carbonate}$	$= 0.083S + 0.307 Cu_{carbonate} - 0.684\ Cd_{exchangeable} + 3.398OM - 219.01$	$R^2 = 0.947, p < 0.001$
$Cd_{Fe-Mn\ oxidising}$	$= 9.344 + 1.1 \times 10^{-5}\ Bacteria_{Total} + 0.001\ SOB$	$R^2 = 0.857, p < 0.001$
$Cd_{organic}$	$= 10.157 + 3.333\ Cu_{organic} - 0.004\ Denitrifier - 0.228\ Cd_{residual}$	$R^2 = 0.807, p < 0.001$
$Cd_{residual}$	$= 81.239 - 0.746\ Cd_{carbonate} - 0.261\ Zn_{exchangeable} - 0.004\ N - 0.863\ Cd_{organic} - 0.530\ Cd_{exchangeable}$	$R^2 = 0.954, p < 0.001$

the Fe–Mn-oxidising, carbonate and exchangeable fractions of Cu in soils G1 and G2 were higher than those of CK. This result is consistent with a previous study. Luo also observed an increase of Fe–Mn-oxidising-bound Cu in wastewater-irrigated soil.[29] Large variations in Cd fractionation were observed, especially for the non-residual fraction. However, no consistent changes were observed among the three soil sites. A high proportion of the exchangeable and carbonate fractions were observed at G1 and especially G2. Comparing with the residual fractions of Cu and Zn, the percentage of residual Cd was relatively low, ranging from 39.64 to 45.23%, 27.07 to 35.71% and 42.78 to 63.38% for soils G1, G2 and CK, respectively.

Multiple stepwise regressions were used to select the significant variables influencing the metal partitioning in the soil of the flooded paddy, and to establish an equation. As shown in Table 2, most of the equation had a high precise ability with a high R^2 value. This indicated that the metal partitioning patterns in the paddy soil were in dynamic equilibrium, but the balance could be modified by three type parameters. The first was the interaction between different metal fractions,

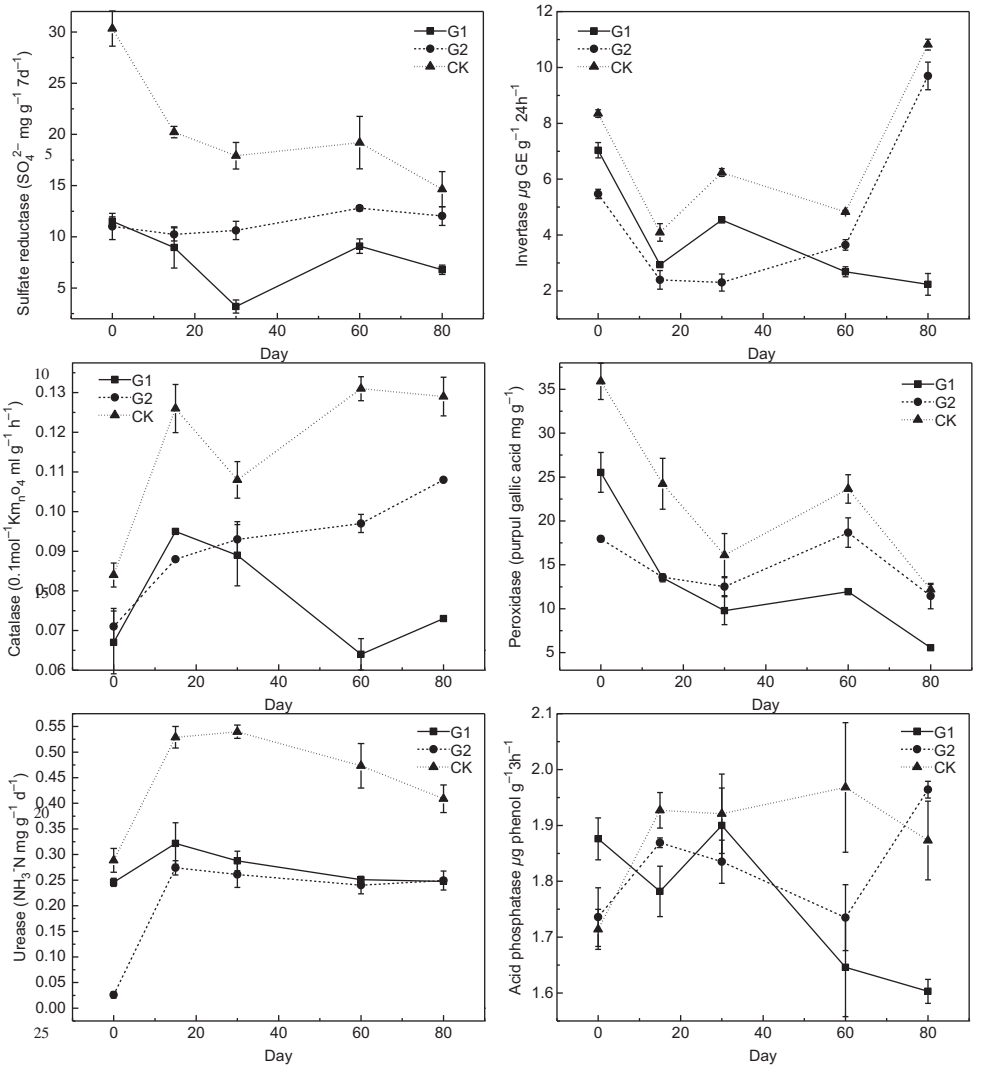

Figure 3. Temporal variations in soil enzyme activities of the flooded paddy soil (mean ± SE).

Table 3. Correlation coefficients (r) for relationships between soil properties and heavy metal contents in different fractions of the paddy soil ($n = 45$, 3 sites × 3 plots × 5 times).

	Zn_{F1}	Zn_{F2}	Zn_{F3}	Zn_{F4}	Zn_{F5}	Cu_{F1}	Cu_{F2}	Cu_{F3}	Cu_{F4}	Cu_{F5}	Cd_{F1}	Cd_{F2}	Cd_{F3}	Cd_{F4}	Cd_{F5}
Sulfate reductase	−0.817**	−0.331	0.282	−0.289	−0.701**	−0.708**	−0.628*	−0.633*	−0.398	−0.534*	−0.633*	−0.432	−0.402	−0.492	−0.806**
Invertase	−0.329	−0.195	−0.079	−0.420	−0.483	−0.154	−0.441	−0.419	−0.229	−0.286	−0.209	−0.335	−0.221	−0.351	−0.367
Catalase	−0.467	−0.293	−0.233	−0.479	−0.645**	−0.657**	−0.574*	−0.541*	−0.347	−0.469	−0.462	−0.391	−0.395	−0.372	−0.639*
Peroxidase	−0.522*	−0.174	−0.284	−0.259	−0.596*	−0.553*	−0.458	−0.410	−0.577*	−0.379	−0.490	−0.246	−0.335	−0.384	−0.599*
Urease	−0.598**	−0.409	−0.385	−0.437	−0.772**	−0.787**	−0.707**	−0.758**	−0.428	−0.745**	−0.756**	−0.563*	−0.502	−0.503	−0.790**
Acid phosphatase	−0.378	−0.381	−0.522*	−0.207	−0.528*	−0.434	−0.517*	−0.451	−0.634*	−0.408	−0.386	−0.344	−0.808**	−0.214	−0.329
Total bacteria	−0.783**	−0.331	−0.015	−0.328	−0.871**	−0.872**	−0.705**	−0.736**	−0.431	−0.646**	−0.761**	−0.460	−0.584*	−0.458	−0.906**
Metal-resistant bacteria	−0.592*	−0.421	−0.019	−0.359	−0.653**	−0.519*	−0.644**	−0.557*	−0.584*	−0.460	−0.436	−0.437	−0.594*	−0.387	−0.539*
Ammonia oxidiser	−0.235	−0.149	−0.019	−0.365	−0.396	−0.475	−0.326	−0.355	−0.371	−0.407	−0.430	−0.215	−0.426	−0.097	−0.286
Denitrifier	−0.109	−0.052	0.363	−0.197	0.232	−0.072	0.054	−0.047	−0.352	−0.061	−0.073	0.009	0.025	−0.167	0.129
Sulfur-oxidising bacteria	−0.329	−0.006	−0.038	−0.135	−0.435	−0.449	−0.242	−0.250	−0.254	0.028	−0.159	0.047	−0.506	−0.004	−0.511
Sulfur-reducing bacteria	−0.366	−0.257	0.468	−0.124	−0.052	−0.096	−0.223	−0.215	−0.275	−0.235	−0.205	−0.243	−0.125	−0.238	−0.089

Note: **Correlation is significant at the 0.01 level (two-tailed, $n = 45$).
*Correlation is significant at the 0.05 level (two-tailed $n = 45$). F1, exchangeable fraction; F2, carbonate fraction; F3, Fe–Mn oxidising fraction; F4, organic fraction; F5, residual fraction.

Table 4. Data from multiple regression analysis between microbial properties, soil physicochemical characteristics and metal concentrations in different forms ($n = 45$, 3 sites × 3 plots × 5 times).

Endpoint	Multiple linear regression	Statistics
Sulfate reductase	$= 17.738 - 0.150\ Zn_{exchangeable} - 4.263\ Cd_{residual}$	$R^2 = 0.766, p < 0.001$
Invertase	All variables are removed	–
Catalase	$= 0.113 - 0.003\ Cu_{exchangeable}$	$R^2 = 0.432, p = 0.008$
Peroxidase	$= 38.740 - 0.024\ Cd_{residual}$	$R^2 = 0.355, p < 0.019$
Urease	$= 0.625 - 0.105\ Cd_{residual} + 0.001$ N	$R^2 = 0.756, p < 0.001$
Acid phosphatase	$= 2.062 - 0.927\ Cd_{Fe-Mn\ oxidising}$	$R^2 = 0.652, p < 0.001$
Total bacteria	$= 1,729,902.3 - 651,583.6\ Cd_{residual}$	$R^2 = 0.890, p < 0.001$
Metal-resistant bacteria	$= 61,741.2 - 38.6\ Zn_{residual}$	$R^2 = 0.427, p = 0.008$
Ammonia oxidiser	$= 3776.3 - 1.811$N	$R^2 = 0.397, p = 0.007$
Denitrifier	All variable are removed.	
Sulfur-oxidising bacteria	$= 175.4$ P $- 45893.9$	$R^2 = 0.884, < 0.001$
Sulfate-reducing bacteria	All variables are removed	

which made large contribution to metal partitioning. The second was the soil geochemical factors, including the pH value, organic matter and the P, S and N contents. These parameters have been previously discussed by many researchers.[25,30–32] The third was the soil microbial parameters. Organic Zn and Fe–Mn-oxidising Cd were easily affected by the soil microbial parameters. The concentration of organic Zn was significantly improved by soil bacteria, which explained 69.8% of the variance in organic Zn. The other three factors influencing the content of organic matter-bound Zn were residual Cd content, residual Zn content and the abundance of denitrifier, contributing 11.9, 12.0 and 3.9%, respectively, to the variance in the equation. The Fe–Mn-oxidising Cd was significantly predicted by the abundance of aerobic or facultative anaerobic bacteria and SOB, explaining 64.7 and 21%, respectively, of the variance.

3.3. *Soil microbial properties*

Soil enzymatic activities may be affected by both the level of substrates and the level of pollutants in soil, thus, the activities of most enzymes were variable during the rice development seasons (Figure 3). The sulfate reductase activity in soils G1 and G2 was significantly lower than that in CK, being one-third or one-half lower than that in CK. Sulfate reductase activity was significantly correlated with the contents of exchangeable Zn ($p < 0.01$), residual Zn ($p < 0.01$), exchangeable Cu ($p < 0.01$), carbonate Cu ($p < 0.05$), Fe–Mn-oxidising Cu ($p < 0.05$), residual Cu ($p < 0.05$), exchangeable Cd ($p < 0.05$) and residual Cd ($p < 0.05$, Table 3). However, sulfate reductase activity was affected most by the contents of exchangeable Zn and residual Cd. Exchangeable Zn and residual Cd explain 66.7 and 9.9% of the observed variance, respectively (Table 4). The mean activities of invertase at soils G1 and G2 was 55.35 and 68.37% lower than that of CK, respectively, but no significant correlation was observed between the invertase activity and metal content. The mean activities of catalase at G1 and G2 was 32.87 and 20.93% lower than that of CK. Catalase activity was significantly negatively correlated with the contents of residual Zn ($p < 0.01$), exchangeable Cu ($p < 0.01$), carbonate Cu ($p < 0.05$), Fe–Mn-oxidising Cu ($p < 0.05$) and residual Cd ($p < 0.01$). However, catalase was affected most by the content of exchangeable Cu, which explained 43.2% of the observed variance (Table 4). The peroxidase activity varied from 5.56 to 35.89 purple gallic acid mg g^{-1} dried soil, which was significantly negatively correlated with the contents of exchangeable Zn ($p < 0.05$), residual Zn ($p < 0.05$), exchangeable Cu ($p < 0.05$), organic Cu ($p < 0.05$) and residual Cd ($p < 0.05$). However, only the residual Cd can be involved in the regression equation, thus explaining 35.5% of the peroxidase activity (Table 4). This means that the reduction in peroxidase was partly caused by the accumulation of residual

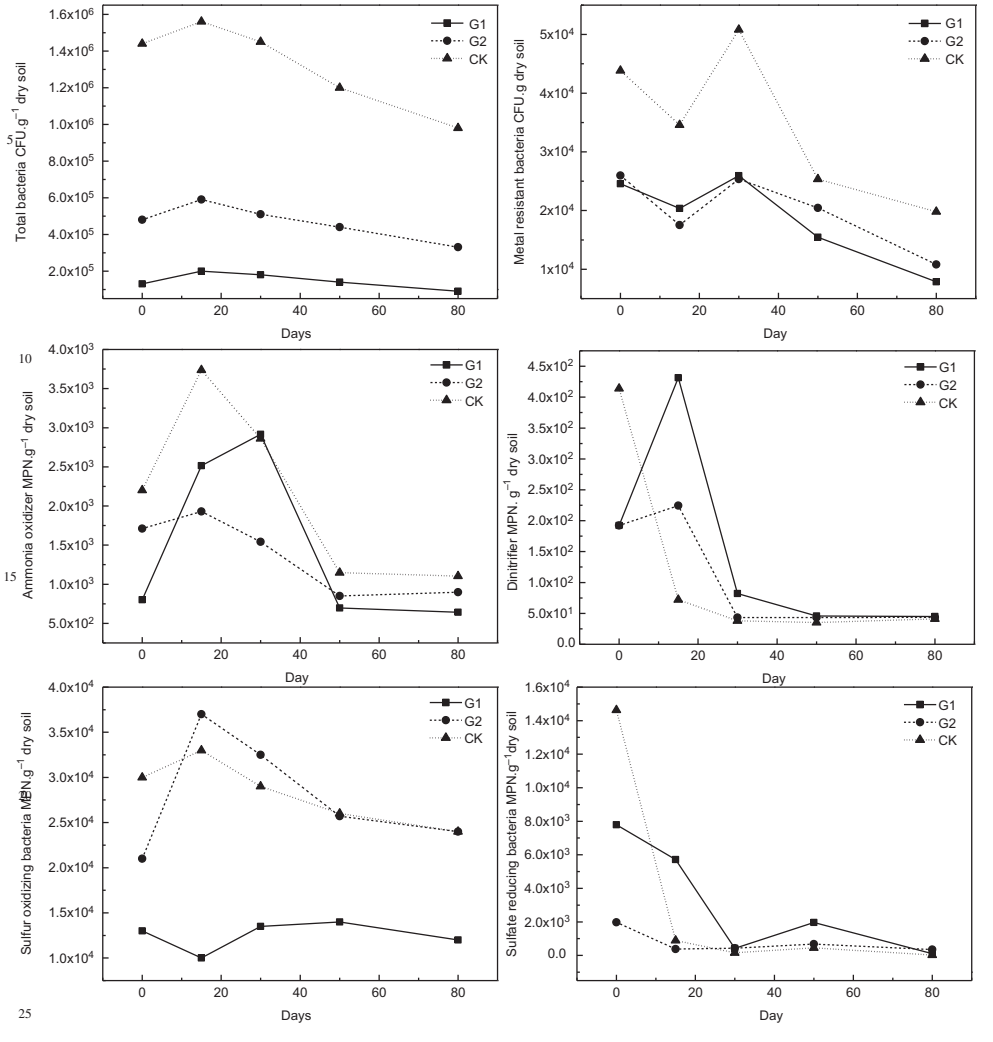

Figure 4. Temporal variations in the abundance of physiological groups in the flooded paddy soil.

Cd. The mean soil urease activities of soils G1 and G2 were 29.51 and 42.63% lower than those of the CK. The soil urease activity was significantly negatively correlated with the contents of exchangeable Zn ($p < 0.01$), residual Zn ($p < 0.01$), exchangeable Cu ($p < 0.01$), carbonate Cu ($p < 0.01$), Fe–Mn-oxidising Cu ($p < 0.01$), residual Cu ($p < 0.01$), exchangeable Cd ($p < 0.01$), carbonate Cd ($p < 0.05$) and residual Cd ($p < 0.05$). However, urease was affected most by the content of residual, which explained 62.4% of the variance (Table 4). These results suggest that the high toxicity of Cd can suppress the urease enzyme activity in paddy soil.[9] The acid phosphatase activities at soils G1 and G2 were relatively stable, and were only slightly lower than those of the control site. Acid phosphatase activity was significantly negatively correlated with the contents of Fe–Mn-oxidising Zn ($p < 0.05$), reside Zn ($p < 0.05$), carbonate Cu ($p < 0.05$), organic Cu ($p < 0.05$) and Fe–Mn-oxidising Cd ($p < 0.01$); Fe–Mn-oxidising Cd explained up to 65.2% of the observed variance in acid phosphatase activity (Table 4).

Generally, there was a lower abundance of microbial physiological groups in soils G1 and G2 than at the control site (Figure 4), but the variations were not the same for the different groups

and soils. Although oxygen was depleted quickly by aerobic bacteria and chemical oxidation reactions in most regions of the rice soil after flooding,[33] many aerobe bacteria remained active in the bulk soil. Overall, the number of total bacteria varied from only 0.9×10^4 to 9.8×10^5 cells per g of dried soil. The abundance of total bacteria in soils G1 and G2 resulted in cell numbers up to one order of magnitude lower than those of CK. The abundance of total bacteria was significantly correlated with the contents of exchangeable Zn ($p < 0.01$), residual Zn ($p < 0.01$), exchangeable Cu ($p < 0.01$), carbonate Cu ($p < 0.01$), Fe–Mn-oxidising Cu ($p < 0.01$), residual Cu ($p < 0.01$), exchangeable Cd ($p < 0.01$), Fe–Mn-oxidising Cd ($p < 0.05$) and residual Cd ($p < 0.05$). However, only the residual Cd can be involved in the equation, explaining ~89.0% of the variation in the abundance of total bacteria (Table 4) meaning that the reduction in total bacteria was mainly due to the accumulation of residual Cd. Soil from the control site had the highest abundance of metal-resistant bacteria prior to transplanting, whereas the abundance of metal-resistant bacteria in sites G1 and soil G2 were nearly equal. The abundance of metal-resistant bacteria was significantly correlated with the contents of exchangeable Zn ($p < 0.05$), residual Zn ($p < 0.01$), exchangeable Cu ($p < 0.05$), carbonate Cu ($p < 0.01$), Fe–Mn-oxidising Cu ($p < 0.01$), organic Cu ($p < 0.01$), Fe–Mn-oxidising Cd ($p < 0.01$) and residual Cd ($p < 0.01$). However, only residual Zn can be introduced in to the equation, explaining 42.7% of the variation in the abundance of metal-resistant bacteria. The abundance of ammonia oxidiser fluctuated between 643 and 2915, 850 and 1929, and 1105 and 3738 cells per g of dried soil for soils G1, G2 and CK, respectively. The abundance of denitrifying bacteria in the bulk soil fluctuated between 40 and 450 cells per g of dried soil, with a maximum of 3738 cells per g of dried soil (CK, 15 days), and a minimum of 643 cells per g of dried soil (G1, 80 days). However, neither ammonia oxidiser nor denitrifying bacteria were significantly correlated with metal content. Low abundances of SOB and SRB were observed in paddy soil. The SOB ranged from 10,000 to 33,000 cells per g of dried soil, whereas the SRB ranged from 18 to 14,634 cells per g of dried soil. Neither SOB nor SRB was significant correlated with metal contents (Table 3).

4. Discussion

The long-term, improper recycling of e-waste has not only resulted in metal accumulation in the studied paddy soil, but has also modified the metal partitioning pattern. Thus, the accumulation of metals may have adverse effects on the soil ecosystem. Lower soil enzyme activities and microorganism abundance were observed in the polluted soils when compared with the control site, which may have contributed to the accumulation of pollutants and the degradation of soil quality. Our results show that sulfate reductase, urease, acid phosphatase and total bacteria were highly sensitive to the accumulation of trace metals in the paddy soil. Invertase was not sensitive to trace metal accumulation in the e-waste contaminated soil, although it has previously been reported as being more sensitive to metal contamination than catalase and phosphatase in soil contaminated with a single metal.[34] This effect may contribute to the interaction of different pollutants.[9] The contents of exchangeable Zn, exchangeable Cu, Fe–Mn-oxidising Cd, residual Zn and residual Cd were parameters that affected the soil microbial properties to the greatest extent, due to the metals in the exchangeable (dissolved) fraction and the carbonate fraction being highly mobile. Metal in these forms usually exerts a strong inhibitory effect on soil microbial and biochemical parameters.[35] However, metal associated with the Fe–Mn oxides showed intermediate mobility. Changes in the redox conditions may cause the release of metal, but some metal precipitates are insoluble if they are present in a sulfide mineral.[36] Although metal in the residual fraction is usually less active, it may play an important role as a pool to replenish metals in the soil solution, particularly Cd. As the most mobile and potentially bioavailable metal, Cd is primarily scavenged by non-detrital carbonate minerals, organic matter, and Fe–Mn oxide minerals.[37]

Soil microorganisms also play a role in the metal portioning of soil. Some microbial processes, including autotrophic and heterotrophic leaching mechanisms, reductive precipitation, sulfate reduction and metal sulfide precipitation, nitrification and denitrification [38] significantly influence metal mobility.[12,39] Furthermore, bacteria can immobilise metals through adsorption by bacterial cell walls.[40] However, this effect was restricted to non-residual fractions, especially for organic matter-bound Zn and Fe–Mn oxides-bound Cd. This is partly consistent with the research of Barrow [41] and Hamon et al.,[42] who believed that microbial-mediated metal partitioning in soils most likely involves an increase in organically bound metal. This may contribute to the adsorption of soil microorganisms to metal ions. Despite only constitute a minor fraction of the total solid mass in the soil, their enormous surface area would have accumulated a considerable part of the soil metal.[43,44] In addition, the rapid oxygen consumption by soil aerobic or facultative anaerobic bacteria would accompany the rapid decrease of Eh in the flooded paddy soil, indicating the onset and continuation of reducing condition. Under reducing condition, Cd may be co-precipitated with sulfide-containing solids or re-adsorbed on secondary iron sulfide precipitates, thus increasing the fraction of Fe–Mn-oxidising Cd. This also indicates that Cd was more sensitive than Zn to Eh changing in the paddy soil.

Nonetheless, modification of the other fractions of trace metal by soil microorganisms was relatively weak, as confirmed by the relatively low contribution to the variance. One possible explanation is that microbial physiological groups were sensitive to several environment factors, hence, their abundance varied at different times under the field conditions. Furthermore, the effect of microorganisms is usually restricts to the microenvironment. However, this effect would be small when compared to the substantial changes in the soil physiochemical characteristics of the soil.

To our knowledge, this is the first in-depth study investigating the detailed chemical fractionation/ partitioning of Cu, Zn and Cd and their relationships with soil microbial properties in paddy soil contaminated by improper e-waste recycling.

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