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From Removal to Immobilisation: A Closed Mass Balance with Multi-Element Internal Controls Reveals the Fate of Chromium in Vermicomposted Tannery Sludge

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Abstract

Vermicomposting of chromium-laden tannery sludge is widely reported to remove 30–96% of the metal, but such figures rest on concentration change alone. We tested this in 60-day mass-closed reactors, comparing composting with vermicomposting by *Eisenia fetida* and *Eudrilus eugeniae*, with and without bamboo biochar. Against a nominal baseline corrected for measured dry-matter loss, the composting control at matched sludge loading recovered 102.7% of the expected chromium and the vermicomposting treatments only 35.4–44.4%. Chromium cannot volatilise or drain from these reactors; the exchangeable plus carbonate-bound pool never exceeded 0.75%, and earthworm biomass could hold at most 0.8% of the missing chromium, so the deficit is not due to removal. Chromium shifted towards the Fe–Mn oxide-bound fraction (72.6–74.4%) in three out of four vermicomposting treatments, consistent with retention in a form that partial acid digestion does not fully liberate, pending confirmation by total decomposition. Across thirteen elements, the deficit was unrelated to biological essentiality, and inert titanium returned 46.9% apparent removal. Hexavalent chromium fell further under vermicomposting (30.6–45.1% of expected) than under composting (55.3–67.8%), most consistently with biochar. Vermicomposting detoxifies and immobilises chromium without removing it; reuse should be judged on mobility, speciation, and total recoverable chromium.

Keywords: tannery sludge; vermicomposting; chromium; mass balance; hexavalent chromium; sequential extraction

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1. Introduction

Tannery leather processing generates approximately 600,000 tonnes of chromium-laden sludge globally each year [1], much of it from facilities in South and Southeast Asia where regulatory capacity for hazardous waste management is limited. Raw tannery sludge typically contains 20,000–50,000 mg/kg of total chromium, along with high concentrations of organic matter, sulphate, sodium, and a range of co-contaminant trace metals [2–4]. Conventional disposal options such as landfills, controlled incineration, and chemical

reduction with $FeSO_4$ followed by alkaline precipitation with $Ca(OH)_2$ are constrained by cost, secondary pollutant generation, or the need for impervious containment, and none recovers value from the waste's embedded organic and nutrient fractions [5–7]. This combination of high volume, hazardous chemistry, and limited treatment infrastructure makes tannery sludge a persistent regional pollution problem for which biotechnological alternatives are urgently needed.

Vermiremediation, the use of earthworms and their associated gut microbiota to transform organic-rich industrial waste into stabilised compost, has been investigated as a low-cost, low-infrastructure alternative for chromium-bearing sludge [8–12]. Bamboo biochar amendment has been shown to improve earthworm survival and metal sorption performance during composting [13–15], and combined earthworm–biochar systems have been reviewed as a route to metal immobilisation rather than extraction [16]. However, the published literature is dominated by total-chromium endpoints, and the mechanistic basis of the reported removal remains poorly resolved.

The attraction of this route is that it treats hazardous waste and generates a resource in a single operation, and the evidence base matters most on the resource side. Vermicompost derived from tannery sludge is proposed for return to land, and the decision to apply it, at what rate, to which crops, and against which regulatory threshold, rests entirely on how much chromium the finished material still carries. That quantity is almost never measured against a mass balance. It is inferred from a reported removal efficiency. A recycling route whose safety case rests on a removal figure therefore inherits whatever that figure actually measures, and establishing what it measures comes before any assessment of the product's suitability for reuse.

Although chromium is the process metal that defines tannery sludge, it is not the only element of concern. The sludge used here also carried substantial zinc, manganese, barium, copper, strontium, nickel, lead, and cadmium, together with percent-level aluminium, calcium, sodium, and sulphur introduced at successive stages of leather processing (Table 1), and several of these carry independent regulatory significance. A treatment evaluated on chromium alone therefore cannot establish that the resulting material is suitable for any downstream use.

Reported total-chromium reductions across the tannery-sludge vermicomposting literature span approximately 30–96% [8–10,12,17], and such figures are routinely interpreted as removal of the metal from the treated material. One recent study, for example, reported a decline from 554.6 to 23.3 mg/kg over 120 days with *Eisenia fetida* and attributed it to bioaccumulation [12]; a fivefold decline in chromium has also been reported for vermicomposted food-industry sludge [18]. The distinction between an apparent reduction in the extractability of a metal and a genuine shift into more stable geochemical fractions has recently been set out as the central interpretive problem for metal immobilisation in soils [19], where it is resolved through the choice of extraction scheme. The present study extends that distinction to the total determination itself. These values are almost universally derived in the same way: an initial and a final concentration are measured on a dry-weight basis, and the proportional difference is reported as removal efficiency [20,21]. The calculation carries an implicit assumption that is rarely tested. Chromium forms no volatile species under the near-neutral, oxidising conditions of a laboratory vermireactor, and without free drainage, there is no pathway by which it can leave the system. Dry-matter mineralisation concentrates non-volatile elements rather than diluting them, so in a mass-closed reactor, the measured concentration of a conserved element should rise, not fall. Where a decline is nonetheless observed, one of three things must be true: the element left the reactor, it was sequestered in an unsampled compartment, or the measured quantity is not the quantity present.

The decreases reported have been attributed to accumulation in earthworm tissue [12,18], to binding by humic and fulvic substances formed during vermicomposting [18], to the utilisation of metals as enzyme co-factors and the suppression of bioavailability by an enlarged microbial community [11,18], to sequestration in chloragogenous tissue and metallothionein binding within the worm [16], to leaching in free-draining systems, and to Cr(VI) reduction by gut-associated microorganisms [8,9,21]. Of these, only leaching exports chromium from the system, and it cannot operate in an undrained reactor; tissue accumulation is limited by the small worm biomass relative to the substrate; and complexation and reduction change the form of chromium, not its quantity. Whether the reported decreases represent removal therefore depends on a mass balance that these studies rarely report.

The distinction matters for the toxicological claim that follows. Total chromium and hexavalent chromium behave differently under mass-balance reasoning. Total chromium is conserved and cannot be destroyed by biological treatment, so a reported decline requires an external sink or an analytical explanation. Cr(VI), by contrast, is chemically destructible: reduction to Cr(III) removes the carcinogenic species without changing total chromium. A treatment that reduces Cr(VI) and immobilises the resulting Cr(III) can be genuinely protective while removing no chromium whatsoever. Combining the two under a single removal-efficiency metric obscures precisely the outcome that matters for environmental health.

The multi-element dimension bears directly on this methodological question. Chromium is one element among many that share the same fate constraints during closed-system treatment: none is volatile, and none can leave a reactor without drainage. If an apparent decline in chromium concentration reflects a real removal process, it should be specific to chromium and to elements with a comparable biological pathway. If it instead reflects the measurement method, it should appear across chemically dissimilar elements and be absent in elements that are analytically and biologically inert. The co-occurring elements in this sludge therefore serve not merely as additional contaminants of interest but as an internal test of the interpretation placed on the chromium data.

The present study was designed to resolve the chemical fate of chromium during vermiremediation rather than to report removal endpoints. We tested two competing hypotheses. If vermicomposting removes chromium (H1), recovery against a closed mass balance should fall below 100%, the deficit should be specific to chromium and to elements with a biological uptake pathway, and it should be matched by chromium in earthworm tissue. If the decline instead reflects a change in chromium's chemical form that reduces recovery by partial digestion (H2), the deficit should appear across chemically dissimilar elements regardless of biological essentiality, be absent from the inert reference element titanium, and coincide with the redistribution of chromium among geochemical fractions. To test them, we (i) constrain apparent concentration changes against a gravimetric mass balance in mass-closed reactors; (ii) quantify Cr(VI) before and after treatment and obtain Cr(III) by the difference; (iii) use sequential geochemical fractionation to determine where residual chromium partitions; (iv) measure chromium in earthworm tissue and in the total recovered earthworm biomass to establish whether worm biomass becomes a secondary contaminant pathway; and (v) evaluate the analytical recovery of the digestion against certified reference materials. Together, these establish which endpoints can legitimately support a reuse decision for a vermicomposted hazardous waste and which cannot.

Table 1. Physicochemical properties of the raw materials (mean ± SD, $n = 3$).

Parameter	Tannery Sludge	Cow Dung	Bamboo Biochar	Soil	*Azolla microphylla*
pH	8.81 ± 0.05	8.95 ± 0.07	8.65 ± 0.04	6.20 ± 0.10	6.29 ± 0.12
EC (dS/m)	6.34 ± 0.02	5.38 ± 0.01	1.98 ± 0.01	0.04 ± 0.03	2.34 ± 0.01
TN (%)	0.88 ± 0.02	0.59 ± 0.14	0.58 ± 0.04	0.07 ± 0.02	3.12 ± 0.01
TP (%)	0.84 ± 0.01	0.29 ± 0.05	0.19 ± 0.10	0.004 ± 0.01	1.00 ± 0.01
OC (%)	17.30 ± 0.30	25.78 ± 0.09	70.2 ± 0.14	1.09 ± 0.02	61.24 ± 0.02
Na (%)	3.19 ± 0.33	0.14 ± 0.00	0.01 ± 0.00	0.0006 ± 0.00	0.69 ± 0.00
Mg (%)	0.84 ± 0.15	0.22 ± 0.00	0.25 ± 0.01	0.004 ± 0.00	0.47 ± 0.01
Al (%)	1.76 ± 0.10	0.16 ± 0.01	0.25 ± 0.02	0.13 ± 0.00	0.02 ± 0.01
K (%)	3.33 ± 0.23	0.93 ± 0.02	1.24 ± 0.00	0.007 ± 0.00	4.29 ± 0.59
Ca (%)	23.28 ± 0.05	0.57 ± 0.03	0.23 ± 0.00	0.007 ± 0.00	0.71 ± 0.02
S-SO_4 (%)	1.21 ± 0.00	0.15 ± 0.00	0.04 ± 0.00	0.004 ± 0.00	0.19 ± 0.00
Ti (mg/kg)	241.96 ± 17.14	29.98 ± 1.09	16.37 ± 2.09	46.41 ± 13.23	2.96 ± 0.01
V (mg/kg)	34.44 ± 15.46	11.06 ± 0.09	3.27 ± 0.42	7.76 ± 3.11	5.87 ± 0.11
Cr (mg/kg)	36,470.55 ± 761.63	36.27 ± 0.10	6.28 ± 1.22	4.15 ± 1.03	7.62 ± 0.04
Mn (mg/kg)	1517.28 ± 221.79	416.23 ± 23.34	129.60 ± 26.54	27.98 ± 5.65	2601.66 ± 64.65
Fe (mg/kg)	11,818.41 ± 1519.96	3243.02 ± 45.76	1826.09 ± 34.24	1621.51 ± 43.12	1310.45 ± 121.81
Cu (mg/kg)	541.89 ± 51.10	9.82 ± 0.43	5.65 ± 1.01	1.75 ± 0.04	7.15 ± 0.03
Zn (mg/kg)	5631.84 ± 31.61	62.62 ± 12.56	33.50 ± 1.23	3.57 ± 0.08	70.62 ± 8.02
Sr (mg/kg)	397.47 ± 16.91	26.97 ± 1.29	7.82 ± 0.06	0.59 ± 0.01	38.46 ± 0.12
Ba (mg/kg)	706.31 ± 13.15	55.60 ± 11.13	107.39 ± 10.09	5.75 ± 0.04	114.07 ± 32.11
Co (mg/kg)	34.01 ± 14.84	1.28 ± 0.01	0.21 ± 0.00	0.42 ± 0.00	2.02 ± 0.04
Ni (mg/kg)	123.89 ± 18.68	1.87 ± 0.03	1.76 ± 0.02	0.64 ± 0.02	1.05 ± 0.00
Mo (mg/kg)	147.96 ± 64.94	0.69 ± 0.00	0.44 ± 0.00	0.14 ± 0.01	3.50 ± 0.01
Cd (mg/kg)	32.77 ± 15.53	0.11 ± 0.00	0.01 ± 0.00	0.001 ± 0.00	0.02 ± 0.00
Pb (mg/kg)	57.20 ± 24.10	3.80 ± 0.32	2.41 ± 0.05	3.78 ± 0.01	0.70 ± 0.01

EC = electrical conductivity; TN = total nitrogen; TP = total phosphorus; OC = organic carbon. Na to Pb were determined by ICP-MS/ICP-OES after USEPA Method 3050B digestion (Section 2.5).

2. Materials and Methods

2.1. Raw Materials and Earthworms

The tannery leather sludge was collected in Mueang Samut Prakan District, Samut Prakan Province, Thailand, as dewatered activated sludge from a leather-processing facility's combined wastewater treatment system. Cow dung was obtained from the cattle yard of the Department of Animal Science, Faculty of Agriculture, Khon Kaen University, Thailand, and used as a bulking agent and a source of start-up energy for the test earthworms. The Plant Protection Service Group, Sa Kaeo Provincial Agricultural Extension Office, Mueang Sa Kaeo District, Sa Kaeo, Thailand, produced the biochar by pyrolysis of bamboo (*Dendrocalamus sericeus* Munro) at 400 °C under limited oxygen (gasifier, Thonburi University, Bangkok, Thailand). *Azolla microphylla* and the two earthworm species used, *Eisenia fetida* (0.30–0.60 g individual fresh weight) and *Eudrilus eugeniae* (1.00–3.00 g), were obtained from the culture collection of the Vermitechnology for Sustainable Agriculture and Environment programme, Department of Soil Science and Environment, Khon Kaen University, Thailand. Earthworm

cultures were maintained on cow manure free of additional pollutants. Only clitellate adults between two months and one year of age were used. Before mixing, all components were air-dried and passed through a 2 mm sieve; a particle-size distribution of the individual components was not determined. The physicochemical properties of all raw materials are given in Table 1.

The present 60-day experiment was conducted independently of, and as a substantive extension of, the 45-day chromium-focused vermicomposting trial reported by Mongchu et al. [17]. Substrate sources, earthworm cultures, and analytical facilities were retained to ensure inter-study comparability; the present study nevertheless differs in treatment duration (60 versus 45 days), experimental design (inclusion of conventional composting controls alongside vermicomposting with two earthworm species), analytical scope, and statistical framework. No data, figures, or tables reported here overlap with those previously published.

2.2. Experimental Design and Reactor Configuration

Substrate proportions are expressed relative to a 1.000 kg reference charge. The bedding comprised soil, cow dung, and *Azolla microphylla* at 40%, 30%, and 20% of that reference on an air-dry mass basis, a ratio established in preliminary trials in our laboratory. Soil serves as a bulking and pH-buffering component and a source of mineral grit for gizzard function; cow dung provides start-up energy for the earthworms; and *Azolla microphylla* contributes nitrogen and lowers the mixture's carbon-to-nitrogen ratio. Vermicompost, biochar, and *A. microphylla* have been shown in combination to raise organic matter and nutrient status while reducing the exchangeable fraction of a metal contaminant in a spiked tropical soil [22], and it is on that basis that the three were used together in the bedding here; no contribution of any single component to metal behaviour is separated or claimed in the present design. Because *Azolla* was applied at an identical proportion of the bedding in every treatment, it does not act as a variable in any comparison reported here and cannot confound the contrasts between composting and vermicomposting, between species, or between biochar-amended and unamended substrates; the present design was not constructed to isolate any contribution of *Azolla* to metal behaviour, and none is claimed.

Treatment 1 received bedding alone (0.900 kg), and treatments 2–4 received the same bedding together with a 0.100 kg amendment: 10% bamboo biochar (T2), 7.5% tannery sludge with 2.5% bamboo biochar (T3), or 10% tannery sludge (T4), each to a total of 1.000 kg. The 10% amendment rate and the 7.5:2.5 sludge-to-biochar ratio were selected on the basis of preliminary trials in our laboratory. Bamboo biochar was included for its reported capacity to sorb metals and to buffer acute metal exposure during vermicomposting, not as a pH adjustment; substrate pH remained between 7.35 and 8.58 in all treatments. All concentrations are expressed per kilogram of the substrate actually present in each reactor. Each combination was prepared in triplicate, giving twelve treatments and thirty-six reactors. Treatments are designated C1–C4 (composting, no earthworms), Vf1–Vf4 (vermicomposting with *E. fetida*), and Ve1–Ve4 (vermicomposting with *E. eugeniae*).

Clitellate adult earthworms were introduced in proportion to substrate mass at a constant stocking density of 30 individuals per kilogram of dry substrate, giving 27 individuals in treatment 1 and 30 in treatments 2–4. Reactors were circular plastic containers (19 cm height; 15 cm base diameter; 30 cm top diameter) with small basal perforations, placed on individual collection saucers. Moisture was maintained at 60–70% by intermittent water addition without exceeding the substrate's water-holding capacity, and no leachate accumulated in the saucers at any point during the 60-day period. Therefore, free drainage did not occur, and the reactors are treated throughout as mass-closed with respect to non-volatile elements. Temperature ranged between 28 and 33 °C. The 60-day

duration lies within the range applied to laboratory vermicomposting of industrial sludges (60–120 days) [11,12,18], extends the 45-day trial on the same sludge [17] to a more advanced stage of stabilisation, and was sufficient for the C/N ratio of the sludge-bearing vermicomposts to fall from 33.3–36.3 to 12.0–18.5 (Figure S2). Under this configuration, the total mass of any non-volatile element within a reactor is fixed for the duration of the experiment, and any apparent change in that mass must be attributed to sampling or analytical effects rather than to export. Representative samples were collected from each replicate at day 0 and day 60, air-dried, and stored in plastic bags pending analysis; the whole of each sample was then ground and sieved for analysis as described in Section 2.4. Earthworms were recovered at day 60 by hand-sorting, counted, washed, and weighed (PA2102, OHAUS Precision Balance, Parsippany, NJ, USA), and cocoons were counted.

2.3. Gravimetric Determination of Dry-Matter Loss

Substrate dry mass was determined gravimetrically at the start and at the end of the experiment. The full reactor contents were recovered, air-dried under ambient laboratory conditions (28–33 °C) to constant weight, and weighed; dry-matter loss is reported as the proportion of initial dry mass not recovered. The same drying procedure was applied at both time points, and the same air-dried material was subsequently ground for physicochemical and elemental analysis. A single determination was made per reactor rather than per replicate.

2.4. Physicochemical Analysis

Raw materials, compost, and vermicompost were ground to pass a 0.5 mm sieve before analysis; material retained on the sieve was ground further until the whole sample passed, so that the entire sample was analysed. pH and electrical conductivity (EC) were determined in a 1:5 (*w*/*w*) water extract using a pH meter (AB33pH-F, OHAUS, Parsippany, NJ, USA) and a conductivity meter (Starter 3100C ST3100C-F, OHAUS, Parsippany, NJ, USA). Total nitrogen was measured following Jackson's method [23], and organic carbon was measured by the Walkley–Black method [24]. Total phosphorus was determined after digestion in HNO_3:$HClO_4$ (2:1) using the spectrophotometric molybdovanadophosphate method at 420 nm (Spectro UV-2550, LABOMED Inc., Los Angeles, CA, USA).

Sub-Sampling and Particle-Size Considerations

The whole of each air-dried sample was ground to pass 0.5 mm (Section 2.4), so no size fraction was excluded from analysis. Because the argument developed in Section 3.1 rests on a comparison of measured concentrations between systems, the representativeness of the 0.1 g sub-samples taken for digestion nevertheless requires explicit consideration. Composted and vermicomposted materials differ in texture: earthworm casts are fine, whereas unprocessed sludge aggregates, mineral grains, and biochar particles are coarser and must be ground further to pass the sieve. If chromium-bearing particles were unevenly distributed in the ground material, and more so under vermicomposting than under composting, a chromium deficit confined to the vermicomposting treatments could in principle arise from sub-sampling alone, without any change in the chemical form of the element.

Titanium constrains this possibility. Titanium occurs predominantly as TiO_2 in discrete refractory oxide and silicate grains as a component of soil and is therefore the element in this dataset most exposed to a sub-sampling bias of the kind described. It is also the element conventionally adopted as a conservative reference in soil mass-balance studies, in which an element assumed immobile provides the tracer against which the gain or loss of every other element is calculated [25], although mobility has been documented under intense tropical weathering [26]. A bias operating differentially between the two systems would be

expected to depress titanium recovery through a lack of reactivity under vermicomposting relative to composting. The effect is small: recovery was 37–41% under composting and 28–38% under vermicomposting (Section 3.6), a difference of about six percentage points, which is an order of magnitude smaller than the sixty-point gap seen for chromium, and the corresponding principal component score showed no treatment effect ($F(11,24) = 0.98$, $p = 0.49$). The test is not exact because, at day 60, chromium is associated with Fe–Mn oxide phases distributed on fine particle surfaces, whereas titanium resides in discrete grains, and the two need not be distributed identically in the ground material. A sub-sampling effect contributing to the absolute magnitude of the deficit therefore cannot be excluded from the present dataset and is stated as a limitation in Section 4; the titanium data do not support a differential effect large enough to generate the observed contrast between systems.

2.5. Elemental Analysis

Trace and major elements (K, Na, Ca, Mg, P, S, Mn, Cr, Fe, Cu, Zn, Al, Co, Pb, Ni, Ti, V, Sr, Ba, Mo, and Cd) were determined after digestion in concentrated (70%) HNO_3 with 30% H_2O_2 following USEPA Method 3050B [27], using a 0.1 g sub-sample taken to a final volume of 50 mL, with heating at 95 °C to complete the digestion of organic matter, repeated addition and evaporation steps, and a final addition of HCl before dilution to volume with high-purity water. Digests were centrifuged and quantified by inductively coupled plasma mass spectrometry (ICP-MS; Agilent ICP-MS QQQ 8800, Agilent Technologies, Tokyo, Japan); phosphorus and sulphur in the same digests were determined by inductively coupled plasma optical emission spectrometry (ICP-OES; Agilent ICP-OES 5110 Synchronous Vertical Dual View, Agilent Technologies, Petaling Jaya, Malaysia). Determinations were performed and validated at the Inorganic Chemistry Laboratory, Public and Environmental Health, Queensland Public Health and Scientific Services, Queensland Health Pathology Queensland, Australia, against an in-house homogenised laboratory material (LHM), analysed over several years by multiple laboratories, and SQC 414, a former proficiency sample from the National Measurement Institute (NMI), Sydney, Australia, verified by the laboratories participating in that programme (Table S2). Concentration data were retained only where values exceeded the respective instrument detection limits.

Total phosphorus was also determined separately at the Department of Soil Science and Environment, Khon Kaen University, after digestion in HNO_3:$HClO_4$ (2:1). Phosphorus and sulphur were additionally measured by ICP-OES in the Method 3050B digests, and those values, being products of the weaker digestion, are reported with the trace-metal dataset and are not interchangeable with the perchloric determination. Potassium was likewise determined by more than one procedure; the two sets are not comparable, and potassium is accordingly not evaluated against the mass balance at any point in this work. The two digestions are therefore not interchangeable. Method 3050B is explicitly not a total digestion: elements bound within silicate structures are not normally dissolved by it, and a hydrofluoric-acid procedure such as USEPA Method 3052 is required where total decomposition is needed [28]. In a direct comparison across sixteen elements in three standard reference materials, aqua regia digestion recovered on average 80% of certified values, versus 94% when hydrofluoric acid was included; chromium is among the elements least completely recovered, with reported recoveries of 23–74% from standard and sewage-sludge-amended soils, attributed to the resistance of insoluble Cr-bearing minerals to aqua regia [29]. Comparative work on sediments confirms that the fraction not released by Method 3050B is associated with silicate phases and with incompletely dissolved Fe and Mn oxides [30].

2.6. Baseline Concentrations and Mass-Balance Calculation

Samples taken at day 0 were drawn from freshly combined mixtures in which the sludge and bedding components had not yet been physically integrated, and the resulting concentrations proved unrepresentative (Section 3.1). Measured day-0 values are reported in full, but nominal initial concentrations were adopted as the quantitative baseline. These were calculated as the mass-weighted sum of the measured concentration of each component material at its proportion within the reactor, giving 3661 mg/kg chromium at 10% sludge loading and 2750 mg/kg at 7.5%. The expected day-60 concentration for a conserved element is the nominal initial concentration divided by the fraction of dry mass retained (Equation (1)), and recovery is the measured day-60 concentration expressed as a percentage of that expectation (Equation (2)):

$$C_{exp,60} = C_{nom,0}/(1 - L) \quad (1)$$

$$\text{Recovery } (\%) = (C_{meas,60}/C_{exp,60}) \times 100 \quad (2)$$

where $C_{nom,0}$ is the nominal initial concentration (mg/kg), L is the proportional dry-matter loss determined gravimetrically for that reactor, and $C_{meas,60}$ is the measured day-60 concentration (mg/kg).

Chromium was additionally determined in each combined substrate before the reactors were filled. Once the components of a given substrate had been combined and homogenised and before that mixture was divided between reactors, triplicate sub-samples of approximately 0.5 g (five times the mass used for the individual reactor samples) were taken from the bulk mixture and digested and analysed as in Section 2.5. These composite determinations returned total chromium concentrations of 13.2 ± 1.1 mg/kg for the bedding mixture, 26.5 ± 0.8 mg/kg for bedding with biochar, 2241 ± 4 mg/kg at 7.5% sludge loading, and 3193 ± 100 mg/kg at 10% sludge loading. Their replicate precision (RSD 0.2–8.1%) is an order of magnitude better than that of the individual day-0 reactor samples (Section 3.1), consistent with the larger sub-sample mass and with sampling the bulk mixture rather than individual reactors. They are used in Section 3.1 as an independent test of the nominal baseline.

2.7. Chromium Speciation [Cr(VI)/Cr(III)]

Chromium speciation was determined separately as hexavalent and trivalent chromium for all initial and final samples. Hexavalent chromium was extracted by alkaline digestion following USEPA Method 3060A [31], modified for health-risk assessment purposes. A 0.1 g sub-sample of air-dried, ground (<0.5 mm) substrate was digested in 0.28 M Na_2CO_3/0.5 M NaOH, with $MgCl_2$ and phosphate buffer (pH 7) added to suppress oxidation of Cr(III), at 90–95 °C for at least 60 min at the solid-to-solution ratio specified in Method 3060A; the digest was made to 20 mL, filtered, and analysed by ICP-MS (Agilent 8800 QQQ, Agilent Technologies, Tokyo, Japan) at the Inorganic Chemistry Laboratory, Queensland Health, and chromium in the alkaline extract corresponds to Cr(VI). Digestion blanks were carried through each batch and subtracted, and the method detection limit, calculated as three times the standard deviation of the blanks, was approximately 5 mg/kg Cr(VI) in the solid. Accuracy was verified against the certified reference material NCSZC71068 (Cr(VI) in soil; China National Analysis Center for Iron and Steel, Beijing, China; certified 68 ± 7 mg/kg), which returned 74.0 ± 22.3 mg/kg (n = 9 across three digestion batches; mean recovery 109%).

Trivalent chromium was determined as the difference between the total chromium from acid digestion and the hexavalent chromium determined on the same sub-sample at the Inorganic Chemistry Public and Environmental Health, Queensland Public Health and

Scientific Services, Queensland Health Pathology Queensland (Table S4). Two consequences follow and are observed throughout: First, the sum of the two species is not an independent determination of total chromium, and the calculated Cr(III) concentration is not used to infer the generation of Cr(III); changes in that column follow arithmetically from changes in the total. Second, the total chromium used for this difference is the Method 3050B determination made at the same laboratory and reported in Table S12a so that both species and the total derive from a single laboratory and a single set of sub-samples. Hexavalent chromium was not determined in samples containing less than approximately 20 mg/kg total chromium, other than the source materials, because at total chromium of 8–27 mg/kg, any Cr(VI) present would lie at or below the detection limit; the six sludge-free treatments (C1, C2, Vf1, Vf2, Ve1, and Ve2) accordingly carry no Cr(VI) value and are reported as not determined. The speciation data are used here only to establish that Cr(VI) declined.

2.8. Sequential Extraction

The geochemical fractionation of chromium was characterised at Khon Kaen University by the five-step sequential extraction procedure of Tessier et al. [32] on 1.0 g sub-samples of air-dried, ground substrate, giving exchangeable (F1), carbonate-bound (F2), Fe–Mn oxide-bound (F3), organic/sulphide-bound (F4) and residual (F5) fractions; reagents and conditions are given in Table S1. Chromium in each fraction was determined by ICP-MS. Day-0 fractionation was determined on each substrate mixture ($n = 3$) and applies to all three systems prepared from it. The five fractions summed to 99.6–100.0% of an independent HNO_3:$HClO_4$ (2:1) total, and the distribution is reported as a percentage of that total. The fractions are operationally defined rather than mineralogically specific, and non-selectivity of the reagents and re-adsorption between steps are recognised limitations of all such schemes [33,34]; the data are therefore interpreted as relative distributions between treatments rather than as absolute speciation (Table S5).

2.9. Earthworm Tissue Digestion and Bioaccumulation Factor

Surviving worms harvested at day 60 at Khon Kaen University were rinsed in deionised water, placed on moist filter paper at 20 ± 2 °C for 24 h to void gut contents, and then rinsed again and gently blotted dry. Worms were weighed, pooled per replicate ($n = 3$), freeze-dried for 48 h, and homogenised to a fine powder. Approximately 0.2 g of dry tissue powder was digested in 5 mL of concentrated HNO_3 plus 1 mL of 30% H_2O_2 in a microwave digestion system (10 min ramp to 180 °C, 15 min hold). Digests were diluted to 25 mL and analysed for Cr by ICP-MS. The bioaccumulation factor (BAF) was calculated as given in Equation (3):

$$\mathrm{BAF} = \mathrm{Cr}_{\text{worm tissue, dry}} / \mathrm{Cr}_{\text{substrate, dry, day 60}} \quad (3)$$

A certified reference material (DORM-4 fish protein, National Research Council Canada, Ottawa, ON, Canada) was processed alongside each digestion batch, with chromium recovery of 90–110% of the certified value.

2.10. Analytical Quality Control for Total Chromium

All determinations in the trace and major element dataset of Section 2.5 were carried out at the Inorganic Chemistry Public and Environmental Health, Queensland Public Health and Scientific Services, Queensland Health Pathology Queensland, using the Method 3050B procedure described in Section 2.5. A single digestion protocol was therefore applied to all twelve treatments at both sampling times, which is what the comparison developed in Section 3.1 requires: the contrast on which the argument rests is between reactors analysed identically, not between methods. Accuracy was established against an in-house homogenised laboratory material (LHM) and the proficiency material SQC 414, carried

through the same procedure as the samples (Table S2); the recoveries obtained are reported and interpreted in Section 3.2. Within-laboratory precision, expressed as the replicate relative standard deviation, was below 5%. No total-decomposition (hydrofluoric acid) determination is available for the day-60 material, and the consequences for interpretation are set out in Section 4. The physicochemical parameters in Section 2.4, the total phosphorus determination in Section 2.5, and the sequential extraction in Section 2.8 were undertaken at Khon Kaen University; Queensland Health determined the total chromium in Section 2.5 and the hexavalent chromium in Section 2.7.

2.11. Bamboo Biochar Characterisation

The elemental composition of the bamboo biochar was determined by energy-dispersive X-ray spectroscopy (EDS) [35]. Elemental abundances are reported as normalised weight percentages of the elements detected. Hydrogen is not detectable by this technique, so the oxygen-to-carbon ratio derived from these data is not equivalent to a van Krevelen ratio obtained by elemental analysis. Specific surface area, surface functional groups, and point of zero charge were not determined.

2.12. Statistical Analysis

One-way analysis of variance (ANOVA) was applied to earthworm growth parameters, chemical attributes, and elemental concentrations at $p < 0.05$, with mean separation by Tukey's HSD test in SPSS version 28 (IBM Corp., Armonk, NY, USA). Data are presented as means ± standard deviations ($n = 3$). Principal component analysis (PCA) and Pearson correlation were conducted in R 4.4.1. PCA used Varimax rotation with Kaiser normalisation [36], retaining components with eigenvalues exceeding unity. Sampling adequacy was confirmed by the Kaiser–Meyer–Olkin measure (0.822 pre-treatment; 0.842 post-treatment) and Bartlett's test of sphericirty, which was significant in both cases ($df = 91$, $p < 0.001$). Principal component scores were extracted at the replicate level and tested by one-way ANOVA with treatment as the fixed factor, followed by Tukey HSD at $\alpha = 0.05$. Component scores and element loadings are presented together as a biplot for each sampling time (Figure 1), with reactors labelled by treatment and 95% confidence ellipses drawn at the level of the three systems ($n = 12$ each, except $n = 9$ for *E. fetida* at day 0). Analyses used the psych, multcompView, and broom packages. Because ordination associations are statistical rather than mechanistic, we also evaluated each retained component against evidence outside the covariance structure. Element loadings were tested by Spearman rank correlation against the measured enrichment of each element between the sludge-bearing and the bedding-only reactors at day 0. Pearson correlations between chromium and the other elements were recalculated as partial correlations controlling for measured calcium, which enters the reactors almost entirely with the sludge (23.28% in the sludge against 0.57% or less in every other component) and therefore directly indexes the sludge content of each sub-sample. Component scores were tested against measured substrate pH, with and without controlling for system. For treatment Vf3, day-0 determinations of thirteen elements (Na, Mg, Al, K, Ca, V, Cr, Mn, Fe, Cu, Zn, Sr, and Ba) were not available as independent measurements and are reported as not determined; analyses that require these day-0 values (the day-0 ordination, the day-0 partial correlations, and the concentration-basis comparisons of Section The Deficit Tracks Geochemical Affinity, Not Biological Essentiality) were therefore made without that treatment.

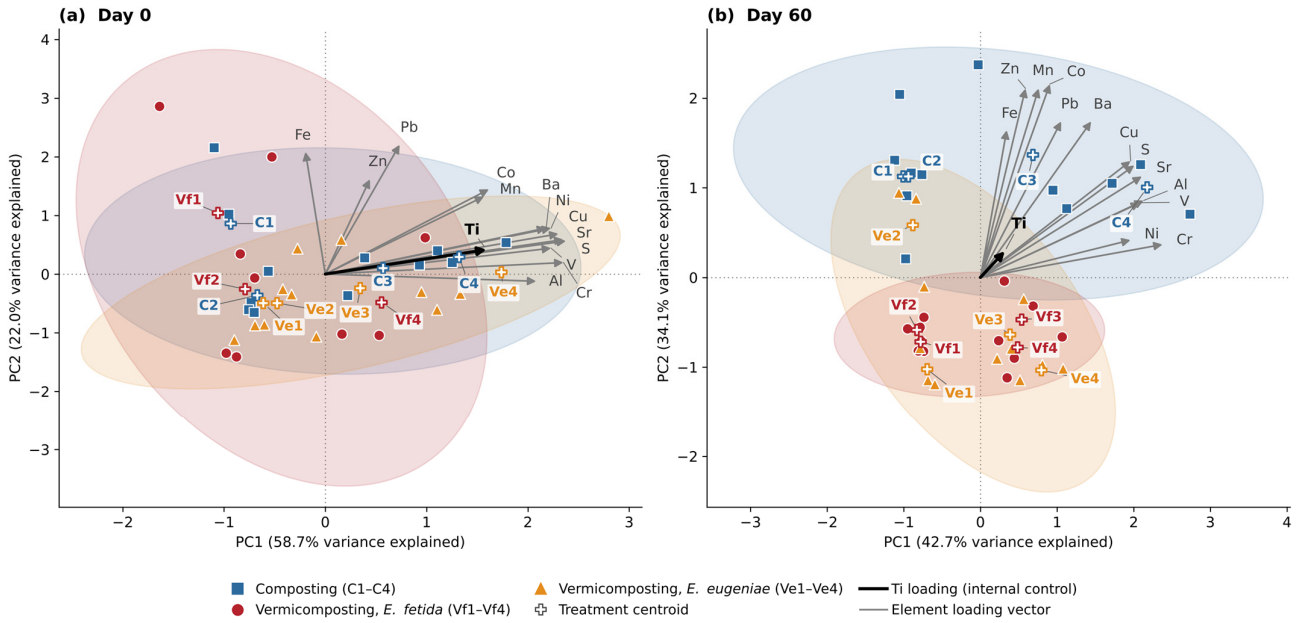


Figure 1. Principal component analysis of fourteen elements (Cr, Mn, Fe, Al, Ti, V, Cu, Zn, Sr, Ba, Co, Ni, Pb, and S) in the reactors at (**a**) day 0 (n = 33; day-0 values for Vf3 not determined) and (**b**) day 60 (n = 36), after autoscaling and Varimax rotation with Kaiser normalisation. Symbols are individual reactors, labelled at the centroid of each treatment; shaded areas are 95% confidence ellipses for the three systems (n = 12 each, except n = 9 for *E. fetida* at day 0). Grey arrows are element loading vectors; the titanium vector, in black, is the internal control. At day 0, the three systems are not resolved. At day 60, composting separates completely from both vermicomposting treatments on PC2, which carries the redox-sensitive elements (Co, Mn, Zn, Ba, Pb, and Fe), while PC1, which carries the elements entering the reactors with the sludge, does not separate them; titanium resolves onto a third component that shows no treatment effect. C = composting; Vf = vermicomposting with *Eisenia fetida*; Ve = vermicomposting with *Eudrilus eugeniae*. Treatments: 1 = bedding only; 2 = bedding with 10% bamboo biochar; 3 = bedding with 7.5% tannery sludge and 2.5% bamboo biochar; 4 = bedding with 10% tannery sludge. Sampling adequacy and the full rotated component matrix are given in Table S6.

3. Results and Discussion

3.1. Mass-Balance Constraints on Apparent Chromium Removal

Measured day-0 chromium concentrations varied widely between reactors of identical composition. The three 10% sludge treatments C4, Vf4, and Ve4 were prepared from the same mixture and differed only in the presence and species of earthworms, yet returned initial concentrations of 5271, 2730, and 5424 mg/kg, respectively, compared to a nominal value of 3661 mg/kg. Titanium, which is geochemically conservative and biologically inert, showed the same pattern: measured day-0 values ranged from 12.7 to 42.2 mg/kg across treatments, with a replicate coefficient of variation averaging 24.5% across treatments and reaching 54% in one case; after 60 days of mixing, this fell only to 19.8%, so the imprecision persists at both time points. Since titanium cannot respond to treatment, this variance is attributable to incomplete homogenisation of freshly combined substrates at the time of initial sampling, and the day-0 dataset is not a reliable quantitative baseline. The multivariate analysis reaches the same conclusion. At day 0, the three systems are not resolved: the confidence ellipses overlap almost completely, and neither component separates them (PC1, $F(2,30) = 1.27$, $p = 0.29$; PC2, $F(2,30) = 0.86$, $p = 0.43$; Figure 1a), which is the expected result for reactors that differ only in the presence and species of earthworms and have not yet been treated but which also confirms that the day-0 measurements carry no usable between-system signal. By day 60, the composting reactors separate completely from

both vermicomposting treatments on the second component ($F(2,33) = 36.76$, $p < 0.001$; Figure 1b). The substitution of nominal values is nonetheless supported for chromium specifically. The mean measured day-0 concentration across the three 10% sludge reactors is 4475 mg/kg, or 1.22 times the nominal value of 3661 mg/kg, so the calculated baseline is not systematically displaced from measurement even though individual reactors scatter widely around it. The nominal values are themselves reproducible from the component analyses: the mass-weighted sum of the measured concentrations in Table 1 returns 3661 mg/kg chromium at 10% sludge loading and 2750 mg/kg at 7.5%. Nominal baselines are used only for those elements where this reconciliation holds; the elements for which it does not are excluded from quantitative treatment and reported separately (Section 3.6; Table S11).

Dry-matter loss differed systematically between systems, averaging 5.7–8.4% in the composting controls and 13.0–20.0% in the vermicomposting treatments, consistent with earthworm-accelerated mineralisation. Evaluated against the nominal baseline and each reactor's measured mass loss, the treatments separate sharply (Table 2, Figure 2); the corresponding figures for all twelve reactors, including the sludge-free bedding and biochar controls, are given in Table S10. The composting control at 10% sludge loading recovered 102.7% of the expected chromium, showing that the nominal baseline, gravimetric measurement, and analytical procedure are mutually consistent to within a few per cent. The corresponding vermicomposting treatments recovered 35.4% and 44.4%, and the two treatments at 7.5% loading recovered 41.6% and 44.2%. Between 55 and 64% of the chromium present in each vermicomposting reactor was therefore not recovered by the digestion (Table 3), in a system from which chromium cannot escape. The deficit is nonetheless earthworm-dependent: it appears only where earthworms were present, and the composting control at matched sludge loading shows no such departure from the mass balance. What follows therefore concerns the chemical form of chromium, not its quantity, and Section 3.3 and Section "The Deficit Tracks Geochemical Affinity, Not Biological Essentiality" develop the role of earthworms in changing that form.

The deficit does not depend on the gravimetric determination. Mineralisation of dry matter can only concentrate a conserved element, so the expected day-60 concentration is bounded below by the nominal initial concentration itself, which corresponds to the limiting case of no dry-matter loss at all. Evaluated against that bound, the assumption most favourable to a closed balance, and one requiring no gravimetric measurement, recovery is 112.1% in C4 against 44.3–51.5% in the four sludge-bearing vermicomposting treatments (Table 2). Incorporating each reactor's measured mass loss sharpens these figures to 102.7% and 35.4–44.4%, respectively, but it does not create the deficit. No physically admissible value of dry-matter loss ($0 \leq L < 1$) can reconcile the vermicomposting measurements with the nominal baseline, since closure would require the reactors to gain rather than lose dry mass over the 60 days. The conclusion is therefore insensitive to the single-determination limitation of the gravimetric measurement noted in Section 4.

An equivalent bound applies to the baseline. For treatment Vf4 to close at 100% on its measured mass loss, the nominal initial concentration would have to be 1297 mg/kg rather than 3661 mg/kg, an overestimate by a factor of 2.82. That, in turn, would require the tannery sludge to contain approximately 12,800 mg/kg chromium rather than the 36,471 mg/kg measured, and the mean measured day-0 concentration across the three 10%–sludge reactors, 4475 mg/kg, is 3.45 times the value such a reconciliation would demand. Neither the gravimetric term nor the baseline term can absorb a deficit of this magnitude.

Table 2. Chromium mass balance in mass-closed reactors. Values are means ± SDs (n = 3).

Treatment	Dry-Mass Loss (%)	Nominal Initial Cr (mg/kg)	Expected Day 60 (mg/kg)	Measured Day 60 (mg/kg)	Recovery (%)	Recovery at Zero Mass Loss (%)
Composting, 7.5% sludge + biochar	6.6	2750	2945	2262 ± 495	76.8 ± 16.8	82.3 ± 18.0
Composting, 10% sludge	8.4	3661	3996	4103 ± 764	102.7 ± 19.1	112.1 ± 20.9
E. fetida, 7.5% sludge + biochar	16.3	2750	3285	1366 ± 368	41.6 ± 11.2	49.7 ± 13.4
E. fetida, 10% sludge	20.0	3661	4576	1621 ± 220	35.4 ± 4.8	44.3 ± 6.0
E. eugeniae, 7.5% sludge + biochar	13.1	2750	3164	1398 ± 239	44.2 ± 7.6	50.8 ± 8.7
E. eugeniae, 10% sludge	13.9	3661	4250	1887 ± 270	44.4 ± 6.3	51.5 ± 7.4

Expected day-60 concentration = nominal initial concentration divided by the fraction of dry mass retained. The final column gives recovery evaluated against the nominal initial concentration alone, i.e., under the limiting assumption of zero dry-matter loss, and is independent of the gravimetric determination. Composting = no earthworms; *E. fetida* and *E. eugeniae* = vermicomposting with each species; 7.5% sludge + biochar = 7.5% tannery sludge with 2.5% bamboo biochar; 10% sludge = 10% tannery sludge. Recovery SD is calculated from replicate day-60 concentrations using the dry-matter loss of each treatment, determined once.

The choice of baseline can be tested directly against those composite determinations (Section 2.6). At 7.5% and 10% sludge loading they gave 2241 ± 4 and 3193 ± 100 mg/kg, or 81% and 87% of the corresponding nominal concentrations, indicating that the calculated baseline modestly overestimates the chromium actually charged to the reactors most plausibly because the sludge sub-sample characterised in Table 1 was slightly richer than the batch used. Substituting the measured composite values for the nominal ones raises every recovery figure but leaves the separation on which the argument rests intact: the composting controls then recover 94.3% and 117.7% of the expected chromium against 40.6–54.2% for the four vermicomposting treatments (Table S13). The conclusion is therefore insensitive to which baseline is adopted. Nominal values are retained as primary because they can be computed for every element on a common basis, whereas the composite determination is available only for chromium.

The two composting controls did not behave the same way. Treatment C4, at 10% sludge loading without biochar, recovered 102.7% of the expected chromium, whereas C3, at 7.5% sludge with 2.5% biochar, recovered 76.8%. The present data do not resolve the reason for this difference; the biochar-bearing substrate is physically more heterogeneous than the sludge bedding matrix and may homogenise less completely on grinding, which would be expected to increase sub-sampling variance, but this is not demonstrated here. The argument developed below is therefore anchored on the contrast at matched sludge loading: C4 against Vf4 and Ve4, which were prepared from a single mixture and differed only in the presence and species of earthworms, giving 102.7% against 35.4 and 44.4%. The 7.5% treatments show the same direction and a comparable gap (76.8% against 41.6 and 44.2%) and are reported as corroboration rather than as the basis of the argument.

Three explanations for this deficit can be excluded. Export with drainage water is precluded by the reactor configuration and, independently, by the sequential extraction data: the combined exchangeable and carbonate-bound pool did not exceed 0.75% of total chromium in any sludge-bearing vermicomposting treatment, placing an upper bound well below 1% on any water-transportable fraction. Retention in earthworm tissue is excluded by direct accounting: even under deliberately conservative assumptions, the recovered earthworm biomass could hold at most 0.2–0.8% of the chromium deficit (Section 3.4). Volatilisation does not apply to chromium unless chromyl chloride is present. Table 3 sets out the chromium inventory of each sludge-bearing reactor in milligrams: 55–64% of the chromium charged to the vermicomposting reactors is unaccounted for, compared with −3% and 23% in the two composting controls, and earthworm biomass accounts for less

than 1% of it. With export, volatilisation, and tissue retention excluded, the remaining candidates are the measurement itself and the representativeness of the sub-samples, which are considered in the following paragraphs and in Section 4.

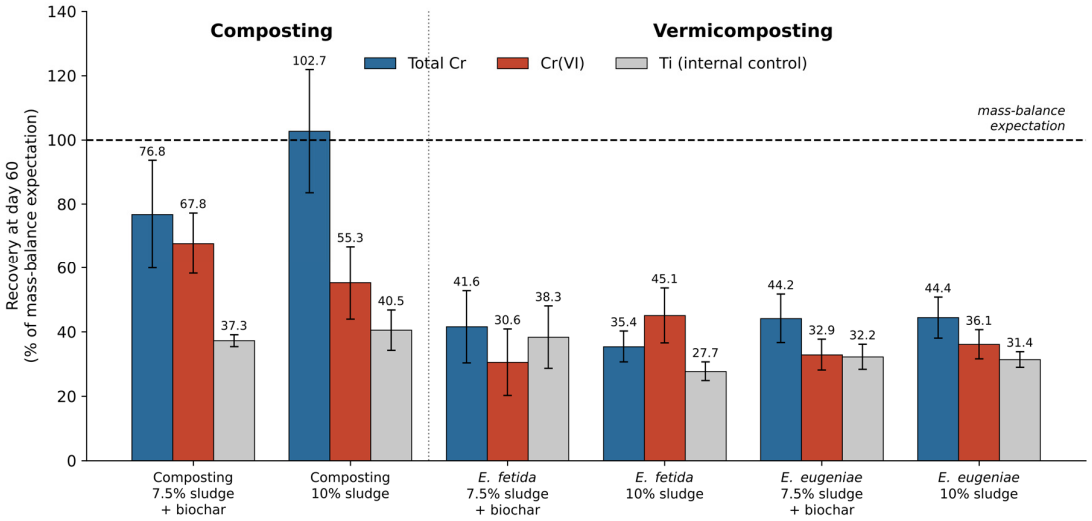


Figure 2. Recovery of total chromium, hexavalent chromium, and titanium at day 60 in the sludge-bearing reactors, expressed as a percentage of the concentration expected from the nominal initial concentration and the measured dry-matter loss of each reactor (Equations (1) and (2)). The dashed line at 100% is the mass-balance expectation for a conserved element. Bars are means ± SDs $n = 3$). Titanium, which is biologically inert and analytically refractory, marks the analytical floor of the method and shows only a small treatment effect; total chromium departs from that floor under composting but converges on it under vermicomposting; Cr(VI), which is chemically destructible, falls furthest under vermicomposting. Treatment 3 contains 7.5% tannery sludge with 2.5% bamboo biochar, and treatment 4 contains 10% tannery sludge. C = composting, Vf = vermicomposting with *Eisenia fetida*, and Ve = vermicomposting with *Eudrilus eugeniae*.

Table 3. Chromium inventory of the sludge-bearing reactors (1.000 kg charge), in milligrams per reactor, calculated from treatment means ($n = 3$).

Treatment	Cr Charged (mg)	Dry Mass Retained at Day 60 (kg)	Cr Measured at Day 60 (mg)	Cr in Earthworm Biomass (mg) [a]	Cr Unaccounted (mg)	Unaccounted (%)
Composting, 7.5% sludge + biochar	2750	0.934	2112	—	638	23.2
Composting, 10% sludge	3661	0.916	3760	—	−99	−2.7
E. fetida, 7.5% sludge + biochar	2750	0.837	1144	≤13.4	1593	57.9
E. fetida, 10% sludge	3661	0.800	1297	≤19.6	2345	64.0
E. eugeniae, 7.5% sludge + biochar	2750	0.869	1215	≤3.4	1532	55.7
E. eugeniae, 10% sludge	3661	0.862	1626	≤3.2	2032	55.5

Cr charged = nominal initial concentration × 1.000 kg; Cr measured at day 60 = measured day-60 concentration (Table 2) × dry mass retained; Cr unaccounted for = charged − measured − earthworm biomass. [a] Upper bound: number of worms recovered × mean adult fresh mass × tissue concentration, with fresh mass treated as dry mass (Section 3.4). Against the measured composite baseline (Table S13), every unaccounted fraction is smaller, but the separation between composting and vermicomposting is preserved.

The nutrient data measured on the same substrates are consistent with this reading but do not, on their own, test it. Total phosphorus and total nitrogen rose in every treatment, as expected of conserved constituents in a reactor losing dry mass. Phosphorus, however, was determined in a different laboratory and by a different digestion (HNO_3:$HClO_4$) from the trace-metal suite, so the comparison cannot isolate the effect of digestion strength and is not used here as independent evidence on the cause of the chromium deficit.

The fractionation data indicate where the chromium resides. Chromium in vermicomposted substrates redistributed into the Fe–Mn oxide-bound fraction, which rose to 72.6–74.4% of total chromium in three of the four sludge-bearing vermicomposting treatments, while the composting controls moved in the opposite direction (Section 3.3; Figure 3). We interpret this as a change in chromium association that makes it less accessible to partial digestion, so the measured concentration falls while the chromium itself remains in the reactor; this interpretation is consistent with the data but has not been verified by a total-decomposition digestion (Section 4). The magnitude of the apparent deficit tracks the intensity of earthworm processing, consistent with earthworm-mediated mineralisation and re-precipitation accelerating this redistribution relative to abiotic composting.

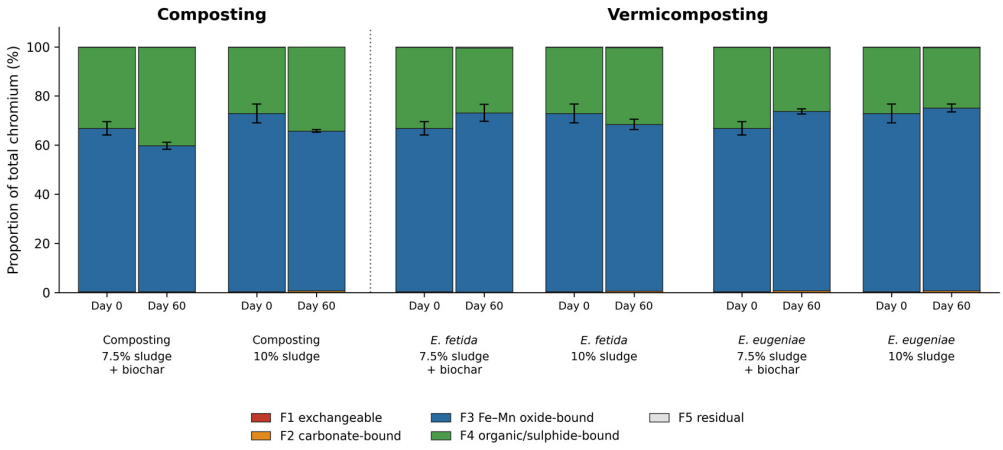


Figure 3. Geochemical fractionation of chromium in the sludge-bearing treatments at day 0 and day 60 (Tessier sequential extraction), expressed as a percentage of total chromium; means of three replicates, with error bars (± SD) on the Fe–Mn oxide-bound fraction. F1 = exchangeable; F2 = carbonate-bound; F3 = Fe–Mn oxide-bound; F4 = organic/sulphide-bound; F5 = residual. Chromium redistributes from the organic/sulphide-bound to the Fe–Mn oxide-bound fraction under vermicomposting in Vf3, Ve3, and Ve4 and in the opposite direction in both composting controls and in Vf4. All columns sum to 99.6–100.0% of the total chromium recovered by the sequential extraction itself; that total is not compared at any point with the Method 3050B determination of Table S12a, with the two being different operationally defined quantities.

3.2. Analytical Recovery on Certified Reference Materials

The reference-material data support this interpretation while also delimiting it. In the same laboratory and by the same protocol, chromium recovery against certified values was 90.7% in the LHM reference material and 96.4% in SQC 414 (Table S2), demonstrating that the digestion does not lose chromium indiscriminately, although recovery by this digestion depends on the sample matrix and on the presence of refractory minerals. Recovery of lithogenic elements in the same materials was markedly lower: titanium 60.4% and aluminium 82.6%, versus 96.5% for iron. The digestion therefore under-recovers elements held specifically in silicate and oxide phases, consistent with the redistribution of chromium observed during vermicomposting (Section 3.3). The reference materials do not carry chromium in that association, so their chromium recovery remains high; the apparent contrast between reference-material performance and reactor mass balance is thus expected

rather than contradictory. Independent confirmation by total-recoverable digestion would nonetheless be required to close the balance directly, and no such determination was made on the day-60 material (Section 4).

3.3. Chromium Speciation and Geochemical Redistribution

Unlike total chromium, hexavalent chromium is not conserved: reduction to Cr(III) removes it. A decline in Cr(VI) is therefore chemically real and is not subject to the mass-balance reduction raised above. Applying the same balance, the composting controls retained 67.8 and 55.3% of the Cr(VI) expected on dry-matter loss alone, whereas the vermicomposting treatments retained 30.6–45.1% (Table 4). The additional loss under vermicomposting is unlikely to be an extraction artefact of the kind proposed for total chromium, since Cr(VI) is determined on a distinct operationally defined pool by alkaline extraction, although occlusion of Cr(VI) within refractory phases cannot be excluded on the present data. Initial Cr(VI) concentrations across the sludge-bearing reactors ranged from 102 to 235 mg/kg and tracked the spread in measured total chromium, as expected, since both determinations were made in the same laboratory on the same sub-samples. This implies reductive transformation: chromium forms no volatile species under these conditions and is retained within the reactor, so hexavalent chromium converted by redox reaction is expected to be present as Cr(III). That inference follows from the mass balance and not from the Cr(III) values of Table S4, which are obtained by difference and therefore carry no information independent of the total (Section 2.7). This is an inference from mass conservation rather than a direct observation; solid-phase spectroscopy would be required to confirm the oxidation state of the retained chromium (Section 4).

Table 4. Hexavalent chromium evaluated against the same mass balance. Unlike total chromium, Cr(VI) can be chemically reduced, so a shortfall in recovery is most consistent with a real transformation rather than an analytical artefact. All values are the Queensland Health determinations described in Section 2.7; means ± SDs ($n = 3$).

Treatment	Cr(VI) Day 0 (mg/kg)	Cr(VI) Day 60 (mg/kg)	Expected if Conserved (mg/kg)	Recovery (%)
Composting, 7.5% sludge + biochar	114.2 ± 26.5	82.9 ± 11.6	122.3	67.8 ± 9.5
Composting, 10% sludge	235.0 ± 142.4	141.8 ± 29.2	256.5	55.3 ± 11.4
E. fetida, 7.5% sludge + biochar	119.3 ± 63.6	43.6 ± 14.7	142.5	30.6 ± 10.3
E. fetida, 10% sludge	102.4 ± 19.5	57.7 ± 10.9	128.0	45.1 ± 8.5
E. eugeniae, 7.5% sludge + biochar	108.8 ± 12.3	41.2 ± 6.0	125.2	32.9 ± 4.8
E. eugeniae, 10% sludge	141.3 ± 54.1	59.2 ± 7.5	163.9	36.1 ± 4.6

Cr(VI) and total Cr are determined on different operationally defined pools: alkaline extraction with an ICP-MS finish (Method 3060A, as modified) and acid digestion (Method 3050B), respectively. Accuracy was established separately for each determination: against the certified reference material NCSZC71068 for Cr(VI) (Section 2.7) and against the in-house homogenised laboratory material and SQC 414 for total chromium (Section 2.10).

Sequential extraction resolves where the residual chromium forms reside, following the approach applied to tannery-impacted soils elsewhere [37]. In sludge-bearing substrates at day 0, chromium was already dominated by the Fe–Mn oxide-bound fraction (F3: 66.5–72.5%) and the organic/sulphide-bound fraction (F4: 27.0–33.0%), with the exchangeable and carbonate-bound pools together below 0.4% and the residual fraction below 0.2%. After 60 days of vermicomposting, F3 rose further in three of the four sludge-bearing treatments, reaching 72.6% in Vf3, 73.1% in Ve3, and 74.4% in Ve4, while F4 declined correspondingly to 24.7–26.4% (Figure 3). Treatment Vf4 is the exception: F3 fell from 72.5 to

67.9% and F4 rose from 27.0 to 31.3%, the same direction as both composting controls, in which F3 declined by 7.4 and 7.1 percentage points. The organic-to-mineral shift is therefore consistent in direction with earthworm processing but is not universal; among the sludge-bearing treatments, it occurs wherever biochar was present and, in its absence, only under *E. eugeniae*. The composting controls are consistent with slower organic-matter turnover and persistent humic binding in the absence of earthworm activity. This redistribution from organic to mineral association is consistent with the mineralisation of organic matter releasing chromium previously bound to humic and fulvic ligands and with co-precipitation of the released Cr(III) with Fe–Mn oxyhydroxides under the neutral to slightly alkaline, oxidising conditions of the vermireactor, although neither step was observed directly. A comparable shift away from the mobile pool has been reported for tannery-sludge vermibeds with *E. fetida*, in which the water-soluble and exchangeable fractions of Cr, Pb, Ni, and Cu fell by 48–69% [11], and UV–visible and FT-IR spectra of vermicomposted industrial sludge have been interpreted as indicating the formation of Cr(III) oxide [18]; those techniques are not element-specific solid-phase probes, however. Where iron is present, the product of such co-precipitation is not a pure Cr(III) hydroxide but a mixed Cr–Fe (oxy)hydroxide in which Cr(III) substitutes for the structurally similar Fe(III); these phases are sparingly soluble and possess only short-range structural order, which is consistent with retention in a digestion-refractory form [38]. Synthesis and solubility work on the same system confirms that, where iron is present, the precipitate is a mixed $Fe_{(1-x)}Cr_x(OH)_3$ phase rather than $Cr(OH)_3$ and that the mixed phase is the more stable of the two [39].

The magnitude of this shift requires comment, because taken at face value, it is smaller than the deficit it is invoked to explain. The Fe–Mn oxide-bound share rose by 1.9 percentage points in Ve4 and by 6.0 points in Vf3 compared to chromium deficits of 56 and 58% on a mass basis. More directly, the F3 association already dominated chromium at day 0 in every sludge-bearing reactor (66.5–72.5%), and the composting control C4 nonetheless recovered 102.7% of the expected chromium by Method 3050B at an F3 share of 72.5%. Method 3050B therefore liberates chromium held in the F3 pool as that pool exists at day 0, and a simple increase in the size of that pool cannot account for the deficit.

The interpretation we propose, which remains to be verified, is not that chromium migrated into a fraction it did not previously occupy but that its association within that fraction changed. At day 0, the sludge-derived chromium is held predominantly as Cr(III) sorbed at the surfaces of pre-existing Fe–Mn oxyhydroxide phases. Mineralisation of organic matter during vermicomposting releases the humic- and fulvic-bound chromium of the F4 pool, and the released Cr(III) co-precipitates with iron and manganese under the oxidising, near-neutral conditions of the vermireactor, becoming occluded within newly formed oxide phases rather than sorbed on their surfaces. The hydroxylamine–acetic acid step of the Tessier procedure dissolves the oxide host itself and recovers occluded and surface-associated chromium alike, which is why the fractionation percentages move only modestly; the nitric–peroxide digestion of Method 3050B, which is not designed to decompose refractory oxide and silicate phases, recovers the second but not the first. On this account, the fractionation percentages register the direction of the transformation while systematically understating its analytical consequence, and the two observations are consistent rather than contradictory.

In the sludge-bearing substrates, the exchangeable plus carbonate-bound pool did not exceed 0.75% of the total chromium recovered by the sequential extraction at any point under vermicomposting, rising only from 0.30–0.36% at day 0 to 0.22–0.73% at day 60. This ratio is computed entirely within the Tessier procedure, is measured within a single sample, and is therefore independent of the Method 3050B determination as well as of both the day-0 heterogeneity and the digestion-recovery issue that affect absolute concentrations,

making it the most robust descriptor of remediation outcome available from this dataset. The composting controls behaved comparably on this measure, the same pool standing at 0.33% in C3 and 0.64% in C4 at day 60. The mobile fraction was therefore low in both systems, and the contrast between them rests on the redistribution of chromium between the organic and the mineral-associated pools rather than on any difference in mobility.

3.3.1. Effect of Bamboo Biochar Amendment

Two endpoints are unaffected by the baseline and digestion issues discussed above: Cr(VI) reduction and the internal distribution of chromium among geochemical fractions. Both are higher in the biochar-amended vermicomposting treatments, although the margin differs between the two earthworm species, and the contrast is not one in biochar alone (Table 5).

Table 5. Effect of bamboo biochar amendment in compost and vermicompost systems. Values are means ± SDs ($n = 3$); cocoons are means of three reactors.

Treatment	Biochar	Cr(VI) Reduction (%)	Change in F3 (Points)	Change in F4 (Points)	Cocoons
Composting, 7.5% sludge + biochar	Yes	27.4 ± 19.7	−7.1 ± 3.1	+7.1 ± 2.4	—
Composting, 10% sludge	No	39.6 ± 38.6	−7.4 ± 3.9	+7.3 ± 2.6	—
E. fetida, 7.5% sludge + biochar	Yes	63.5 ± 23.0	+6.0 ± 4.4	−6.6 ± 2.1	31.0
E. fetida, 10% sludge	No	43.6 ± 15.2	−4.6 ± 4.4	+4.3 ± 2.9	42.3
E. eugeniae, 7.5% sludge + biochar	Yes	62.2 ± 7.0	+6.6 ± 2.9	−7.0 ± 2.1	0.3
E. eugeniae, 10% sludge	No	58.1 ± 16.9	+1.9 ± 4.2	−2.3 ± 2.6	0.3

Treatment 3 contains 7.5% sludge with 2.5% biochar; treatment 4 contains 10% sludge without biochar. F3 = Fe–Mn oxide-bound; F4 = organic/sulphide-bound. Cr(VI) reduction is calculated on the concentration basis, as 100 × (day-0 − day-60 concentration) ÷ day-0 concentration, and is therefore not the same quantity as the mass-balance recovery of Table 4; it is used here only to compare biochar-amended with unamended treatments, and Cr(VI), unlike total chromium, is chemically destructible. The two sets differ in sludge loading as well as in biochar, so the comparison reflects amendment regimes rather than an isolated biochar effect. SDs of Cr(VI) reduction and changes in F3 and F4 are propagated from the day-0 and day-60 replicate values.

Cr(VI) reduction in biochar-amended vermicomposting reached 63.5% for *E. fetida* and 62.2% for *E. eugeniae*, compared with 43.6% and 58.1%, respectively, in the corresponding treatments without biochar. The margin is therefore substantial under *E. fetida* and slight under *E. eugeniae*, and in the composting controls, it runs in the opposite direction: 27.4% with biochar against 39.6% without. Cr(VI) reduction alone accordingly does not establish a general effect of biochar, and the fractionation endpoint is the more consistent of the two. The same treatments also produced the only consistent organic-to-mineral redistribution observed: the Fe–Mn oxide-bound fraction rose by 6.0 and 6.6 percentage points in Vf3 and Ve3, while the organic/sulphide-bound fraction fell by a corresponding 6.6 and 7.0 points, whereas the unamended treatments were inconsistent between species, and the composting controls moved in the opposite direction in both.

This differs from the interpretation that a total-chromium concentration would support. Evaluated on apparent total-chromium removal, biochar appears beneficial for *E. fetida* and detrimental for *E. eugeniae*, and this species-by-amendment interaction has been reported previously [17]. On the endpoints that survive the mass-balance analysis, the interaction is not reproduced in that form: redistribution towards the mineral-associated pool occurs under biochar in both species, and Cr(VI) reduction is greater under biochar in both, although only marginally so in *E. eugeniae*. The sharp species-by-amendment reversal is therefore a property of total-chromium concentration rather than of the endpoints that measure transformation, and it further illustrates the principal argument of this paper. The present data do not identify the mechanism; biochar is known to carry electron-donating surface

functionality and persistent free radicals capable of abiotic Cr(VI) reduction, as well as to sorb metals directly [16,19,40,41], but the solid-phase characterisation required to attribute the observed effect to either pathway—X-ray photoelectron spectroscopy or X-ray absorption spectroscopy—was not performed here, and the mechanism is left open. A parallel amendment study on cadmium-spiked tropical soil, in which biochar was applied together with vermicompost and *Azolla microphylla*, likewise found the combined amendment to outperform each component applied alone on metal-immobilisation endpoints [22].

3.3.2. Bamboo Biochar Composition

The bamboo biochar was dominated by carbon at 84.9% by weight, with oxygen at 10.9% and the remaining 4.0% distributed among silicon, potassium, copper, magnesium, calcium, iron, and aluminium (Table S15). The corresponding atomic oxygen-to-carbon ratio of 0.096 is low and indicates extensive carbonisation and a highly aromatic structure, consistent with the pyrolysis conditions applied [42].

This composition bears on the mechanistic interpretation. A carbon with an oxygen-to-carbon ratio this low carries comparatively few oxygen-containing surface functional groups [42], so electron transfer from carboxyl, phenolic, and quinone functionality is unlikely to be the dominant pathway for the enhanced Cr(VI) reduction observed in the biochar-amended treatments. Physical sorption, as well as electron transfer mediated by persistent free radicals characteristic of highly aromatic biochars, are the more probable contributors. The present data do not distinguish between these; specific surface area, surface functional groups, and point of zero charge would be required, so the mechanism remains open (Section 4).

3.4. Bioaccumulation and Earthworm Performance

Bioaccumulation factors at day 60 were below unity in every treatment, classifying both species as chromium excluders rather than accumulators. A consistent species difference remained: *E. fetida* returned factors of 0.09–0.44 (mean 0.31), compared with 0.07–0.23 (mean 0.12) for *E. eugeniae*. Because these values are ratios of tissue-to-substrate concentration measured at a single time point, they are unaffected by the baseline and mass-balance issues discussed above. Because a ratio below unity does not by itself bound the mass of chromium held in the worms, the chromium burden of the recovered earthworm biomass was also calculated for each sludge-bearing reactor as the number of worms recovered at day 60 (adults and juveniles; Table S7) multiplied by the measured mean fresh mass of the adults and by the tissue concentration (BAF × substrate chromium at day 60). Fresh mass was treated as dry mass, and juveniles were assigned the adult mass, so the estimate is an upper bound. The burden was at most 13.4 and 19.6 mg Cr per reactor under *E. fetida* (7.5% and 10% sludge; 23.4 and 27.5 g of worms) and 3.4 and 3.2 mg under *E. eugeniae* (20.1 and 21.0 g), against chromium deficits of 1535–2364 mg, i.e., 0.2–0.8% of the missing chromium; at a typical tissue dry-matter content of about 16%, the burden falls below 0.15%. Earthworm biomass therefore does not constitute a meaningful sink for chromium, and tissue retention cannot explain the mass deficit in Section 3.1. This contrasts with an interpretation placed on comparable data elsewhere, in which a 96% decline in total chromium was attributed to bioaccumulation by *E. fetida* [12]. This is a favourable outcome for the treatment rather than a limitation of it. Chromium accumulated in harvested worm biomass would create a secondary contaminant burden requiring its own disposal route and would preclude using the worms as a protein resource.

The two species responded very differently to the substrate (Table S7). *Eisenia fetida* populations increased in every treatment, by 31.1 to 54.4% of the number stocked, with the largest increase and the highest cocoon production (42.3 per reactor) in the treatment

carrying the full 10% sludge load. *Eudrilus eugeniae* populations declined in every treatment, by 27.8 to 55.6%, and lost individual mass in all cases.

The decline in *E. eugeniae* cannot be attributed to metal exposure. It was most severe in the sludge-free bedding control (−55.6% in number and −18.5% in mass) and least severe in the treatment carrying the full sludge load (−36.7% and −8.6%). A toxic response would produce the opposite ordering. The pattern is more consistent with the bedding composition or the incubation temperature range of 28–33 °C being suboptimal for this species under the conditions applied, and it indicates that *E. eugeniae* was under general physiological stress independent of the contaminant.

Despite these opposite demographic trajectories, both species remained active for the full 60 days and produced closely comparable dry-matter loss and chromium mass-balance deficits, indicating that the redistribution of chromium is not sensitive to earthworm population size across the range observed here.

3.5. Substrate Properties Governing Chromium Partitioning

Chromium mobility in organic-rich substrates is controlled by more than pH and sulphur. Organic matter (carbon) content determines the size of the ligand pool available to complex Cr(III); electrical conductivity indexes the ionic strength that governs competition for exchange sites; and the concentrations of competing cations and of phosphate influence both sorption and the formation of secondary precipitates. All of these were measured alongside the chromium determinations and are reported in full in Table S3 and Figure S2.

Substrate pH remained between 7.35 and 8.58 throughout, with vermicomposting treatments stabilising towards neutrality (7.35–8.40) and composting controls remaining more alkaline (8.26–8.58; $p < 0.01$). Across this range, Cr(III) hydrolysis and precipitation are strongly favoured, and the exchangeable pool is minimal, consistent with F1 + F2 remaining at or below 0.75% in the sludge-bearing vermicomposting treatments. Sulphur declined significantly in all treatments, most in the *E. eugeniae* sludge-bearing beds, and correlated strongly with chromium across the full dataset (Pearson $r = 0.90$), consistent with both entering the system as chromium sulphate.

Electrical conductivity fell significantly in all treatments ($p < 0.01$), with larger reductions under vermicomposting, and all final values remained below the 4 mS/cm threshold conventionally applied to soil amendments. In a mass-closed reactor, a decline in conductivity cannot reflect the export of soluble salts; it indicates the transfer of ions from solution into complexed or precipitated forms. The conductivity trend therefore parallels the fractionation result rather than being independent of it.

Organic carbon declined significantly in all treatments, with the larger reductions under vermicomposting, while total nitrogen rose 1.05–1.46-fold under vermicomposting against 1.04–1.33-fold under composting. The C/N ratio fell correspondingly, most steeply in Vf3 (66.9%) and Vf4 (57.2%). This transformation bears directly on the fractionation result: mineralisation of the organic pool is expected to release chromium previously held as humic and fulvic complexes, and such release is consistent with the observed transfer from F4 into F3. The magnitude of the shift under vermicomposting, as well as its reversal under composting, tracks the difference in organic-matter turnover between the two systems.

In the correlation matrix, chromium grouped with copper, vanadium, nickel, strontium, cobalt, manganese, sodium, calcium, magnesium, phosphorus, total nitrogen, and organic carbon at $r = 0.85$–0.99. This association is obvious, and the dataset permits the point to be tested rather than assumed. Measured calcium enters these reactors almost entirely with the sludge, at 23.28% in the sludge versus 0.57% or less in every other substrate component, and therefore indexes the sludge content of each sub-sample. After controlling for it in the day-0 sub-samples ($n = 33$), the coefficients for copper, sulphur, nickel, strontium,

barium, and titanium range from −0.31 to 0.11, and none remains significant. Exceptions persist. Vanadium retains a positive partial correlation with chromium ($r = 0.60$, $p < 0.001$), manganese and cobalt take significant negative values (−0.72 and −0.61, $p < 0.001$), and lead has a weaker negative value (−0.32, $p = 0.07$). The shared sludge origin therefore accounts for most of the group but not all of it: vanadium behaves as though associated with chromium beyond their common origin, while the negative values for the three redox-sensitive elements are consistent with control by redox-dependent oxide formation rather than sludge provenance. Those three elements load on the second component (Figure 1), so what remains once the shared sludge origin is removed is the structure the ordination itself resolves. Nominal sludge dose is not an adequate control for this purpose because it takes three values only while the sludge content of individual sub-samples varied substantially at identical nominal loading (Section 3.1). The correlation structure establishes the coherence of the sludge-derived signature, which principal component analysis resolves into a single axis and which the mass-balance analysis then evaluates element by element.

3.6. Parallel Behaviour of Co-Occurring Elements

Because the nominal baseline is only as reliable as the component analyses from which it derives, we first screened elements by reconciling the calculated initial concentration against the mean measured day-0 value in the 10% sludge reactors. Eight elements reconciled within ±30% (Figure 4) and are treated quantitatively; the remainder are reported qualitatively (Section 4).

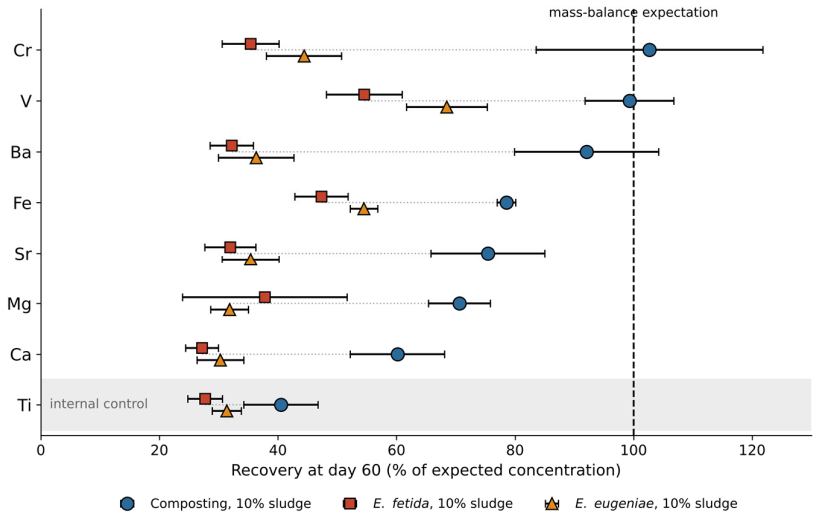


Figure 4. Recovery of eight elements at day 60 against the mass-balance expectation, for the eight elements whose calculated baseline reconciles with the measured day-0 concentration within ±30%. Circles are the composting control at 10% sludge loading (C4); squares and triangles are the vermicomposting treatments at the same loading with *E. fetida* (Vf4) and *E. eugeniae* (Ve4). Error bars are ± SDs ($n = 3$). Recovery is the measured day-60 concentration expressed as a percentage of the concentration expected from the nominal initial concentration and each reactor's measured dry-matter loss (Equations (1) and (2)). The dashed line at 100% is the expectation for a conserved element. Titanium, shaded, is biologically inert and analytically refractory and serves as an internal control; it is the only element showing little separation between systems. Underlying element-by-element values are given in Table S9.

The full accounting is as follows: Twenty-one elements were determined (Section 2.5). Potassium and phosphorus are set aside from this screening: total phosphorus was determined by the stronger HNO_3:$HClO_4$ digestion and is not comparable with the Method 3050B suite, and potassium was determined by two procedures whose results are not comparable

(Section 2.5); sulphur is reported with the substrate properties in Section 3.5 and Table S3. Of the remaining eighteen, eight reconciled within ±30% and are treated quantitatively here (Figure 4), nine did not and are reported without removal-efficiency interpretation (Table S11), and one—cadmium—could not be quantified at all, its blank-corrected concentration in the reactor digests lying at or below the determination limit in every treatment and returning a negative value in 45 of the 72 sub-samples analysed. Cadmium and lead are toxic in all forms and carry independent regulatory significance in sludge-application frameworks at concentrations well below those present in this sludge, and the fact that neither can be evaluated against a closed mass balance here is a material constraint on any statement about the suitability of the treated material, to which Section 3.7 returns.

The pattern established for chromium holds across the subset. Vanadium and barium, which the source-apportionment analysis places with chromium in the sludge-derived component, are close to 99% and 92% under composting, respectively, and fall to 55–69% and 32–36% under vermicomposting. Iron, magnesium, and calcium, which have larger bedding contributions, show partial closure under composting and the same directional gap. Titanium alone shows only a small treatment effect: recovery is 37–41% under composting and 28–38% under vermicomposting. The element-by-element figures underlying this comparison are given for all six sludge-bearing reactors in Table S9. Recovery is low in absolute terms and lower than the 60.4% recovered from certified titanium in the LHM reference material. The present data do not resolve this difference. The reference materials are certified against total decomposition, and the discrete refractory TiO_2 grains that carry most of the titanium in the reactor substrates may be attacked even less completely by Method 3050B than the titanium in the reference material; other contributions cannot be excluded. Whatever its cause, the discrepancy is only weakly treatment-dependent: mean titanium recovery was 38.9% under composting compared to 32.4% under vermicomposting, a difference that is statistically detectable (Mann–Whitney test on replicate-level values, $n = 6$ composting and $n = 12$ vermicomposting; $p = 0.015$) but an order of magnitude smaller than the corresponding gap for chromium, and the corresponding principal component score shows no treatment effect ($F(11,24) = 0.98$, $p = 0.49$; Table S6, Figure S1). The rotated solution places titanium consistently with this role. Before treatment, it loaded on the same component as the sludge-derived elements (PC1, 0.65), as expected when every element in the dataset varies with sludge dose. After 60 days, it resolved onto an independent third component (PC3, 0.88, against 0.13 on PC1 and 0.12 on PC2), while the sludge-derived elements remained together on PC1; that component shows no separation between systems ($F(2,33) = 0.19$, $p = 0.83$). The separation is the multivariate expression of the observation the mass balance makes element by element: the measured concentrations of the other elements moved with treatment, titanium did not, and it therefore ceased to share their covariance structure. Titanium is therefore used here as a null control on differences between treatments, which is the role the argument requires of it, and not as an absolute benchmark of digestion completeness. The reconciliation ratio of its calculated baseline against the mean measured day-0 concentration in the 10%–sludge reactors was 0.70, a deviation of 29.6% and therefore within, though close to the margin of, the ±30% screening criterion applied in this section. No element recovers well under vermicomposting and poorly under composting, which is what a genuine biological removal mechanism would be expected to produce for at least some elements and does not.

Read together, titanium and chromium bracket the interpretation. Titanium shows what a purely analytical deficit looks like: low recovery everywhere and no treatment effect. Chromium shows the pattern expected of a treatment-dependent redistribution: near-closure under composting, a large deficit under vermicomposting, and a corresponding

shift into the Fe–Mn oxide-bound fraction. The remaining elements fall between these end members in proportion to their association with the sludge-derived component.

Sodium warrants separate comment because it was the element most strongly depleted under vermicomposting and because sodicity is a principal agronomic constraint on tannery-sludge reuse. Unlike the transition metals, sodium remains predominantly in soluble and exchangeable form and does not partition into oxide phases, so the redistribution mechanism proposed above cannot account for its behaviour. Its calculated baseline also reconciles poorly with measurement, and no leachate was recovered from the reactors. The apparent sodium depletion is therefore not securely interpretable from the present dataset, and any attribution of these losses to leaching is withdrawn. Pore-water sampling and an ion balance would be required to establish whether sodium was genuinely redistributed within the reactor or whether the difference is a sampling effect.

The Deficit Tracks Geochemical Affinity, Not Biological Essentiality

The elements determined here also divide on a criterion independent of the geochemical one applied above. Copper, zinc, manganese, iron, cobalt, and nickel are essential micronutrients for which earthworms possess regulated uptake, storage, and excretion pathways, including metallothionein binding and chloragosome sequestration [16,43,44]; chromium, aluminium, titanium, vanadium, strontium, barium, and lead have no such pathway and are handled, if at all, by non-specific sorption. This division generates a test that the geochemical classification cannot provide. If the apparent deficits described above arise from biological processing—uptake into tissue or biologically mediated transformation of the metal pool—they should be organised by essentiality because essentiality determines whether a biological pathway exists at all. If instead they arise from progressive redistribution into digestion-refractory mineral phases, essentiality is irrelevant, and the deficits should instead track geochemical affinity.

The data do not support the first alternative, and they do not support it in any of the three sludge-bearing vermicomposting treatments with day-0 data. Figure 5 ranks thirteen elements by apparent removal in treatment Ve4, calculated on the conventional concentration basis. Essential and non-essential elements are interleaved across the entire range. Aluminium, for which no biological uptake pathway exists, returns the largest apparent deficit of any element (72.3%), exceeding both copper (66.7%) and zinc (64.3%), the two elements with the best-characterised earthworm accumulation pathways; iron, an essential micronutrient, returns the smallest (33.5%). Group means differ by 1.3 percentage points—57.3% for the essential elements versus 56.0% for the non-essential—and the two groups are statistically indistinguishable (Mann–Whitney $U = 19.0$, $p = 0.84$).

Repeating the comparison in the remaining two sludge-bearing vermicomposting treatments returns the same outcome (Table 6). No treatment separates the two groups ($p = 0.45$–0.84), and the sign of the difference is not consistent between treatments: the essential group carries the larger mean deficit in Ve3 and Ve4 and the smaller one in Vf4. The rank positions behave similarly. Aluminium holds the largest deficit of the thirteen in all three treatments; iron ranks tenth to thirteenth in all three; and copper and zinc sit in mid-table throughout, at ranks three to six. Nickel essentiality is established in plants and bacteria but not in annelids, and cobalt is required only as a constituent of vitamin B12; reclassifying both as non-essential leaves every comparison unchanged ($p = 0.71$–0.83). The multivariate structure supports the same conclusion by a different route and does so through a positive result rather than a failure to reject. The rotated component matrix at day 60 (Table S6) assigns chromium, aluminium, vanadium, strontium, nickel, and copper to PC1 (loadings 0.81–0.98) and manganese, cobalt, zinc, barium, lead, and iron to PC2 (0.68–0.89). Neither component corresponds to the essential/non-essential division: elements with a

regulated earthworm uptake pathway and elements with none load together on both. What the components do separate is geochemical association, and it is the second component, carrying the redox-sensitive group, that distinguishes the two systems ($F(2,33) = 36.76$, $p < 0.001$), while the first, carrying the elements that entered the reactors with the sludge, does not ($F(2,33) = 0.50$, $p = 0.61$). Where the essentiality test establishes only that the deficits are not organised by biological demand, the component structure indicates what they are organised by instead. As with the correlation structure discussed in Section 3.5, the first component should not be read causally: all sludge-derived elements co-vary with sludge loading across the treatment series, so PC1 principally encodes dose rather than any mechanistic dependence, and the argument advanced here rests on which elements fail to separate along the essentiality criterion rather than on the identity of PC1 itself. Biological essentiality therefore has no detectable organising effect on the deficit, in any treatment and under either classification, which is what the redistribution interpretation predicts and what a biological removal mechanism does not.

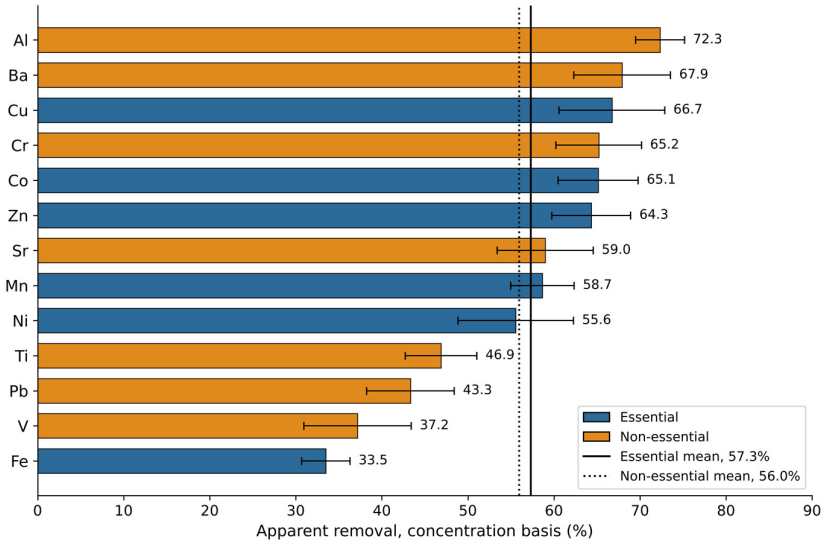


Figure 5. Thirteen elements ranked by apparent removal in treatment Ve4 (10% tannery sludge, vermicomposting with *Eudrilus eugeniae*), calculated on the conventional concentration basis, against biological essentiality. Error bars are ± SDs of the three replicate day-60 concentrations relative to the mean day-0 concentration. Values are used for rank comparison only and are not asserted as quantitative removal (Section "The Deficit Tracks Geochemical Affinity, Not Biological Essentiality"). Essential and non-essential elements are interleaved across the full range, and the two group means are separated by 1.3 percentage points (Mann–Whitney $U = 19.0$, $p = 0.84$). Underlying concentrations are given in Table S12a–d.

Titanium supplies the corresponding positive control, and calculating it as removal efficiencies are conventionally calculated exposes the artefact directly. Titanium can neither be assimilated by earthworms nor exported from a mass-closed reactor, so its true removal is zero by construction. On the concentration basis, it nonetheless returns 46.9% apparent removal in Ve4 and between 5.1 and 57.1% across the three vermicomposting treatments with day-0 data; on the mass basis, which is unaffected by the day-0 heterogeneity, it returns an apparent loss of 61.7–72.3% under vermicomposting and 59.5–62.7% under composting (Table S9). The spread of the concentration-basis values indexes noise introduced by unrepresentative day-0 sampling; the magnitude of the mass-basis values indexes incomplete digestion. Neither quantity has any biological content, and both are of the same order as the deficits reported for chromium and its co-occurring elements. An apparent removal

of this size in an element that cannot have lost a single milligram is the clearest available demonstration that the conventional calculation measures something other than removal.

Two further features of the dataset point the same way. Lead concentrations increased under composting (C3 +10.8%; C4 +14.4%), as did sodium, which is the expected behaviour of conserved elements in a reactor losing dry mass, whether the element is held mainly by organic complexation (lead) or by cation exchange (sodium). Separately, the apparent removal of nickel that also complexes with organic matter was high in the sludge-free bedding reactors—56.2% in Vf1 and 44.3% in Ve1—where nickel is present only at background concentrations of a few milligrams per kilogram. Nickel also returns an apparent gain of 3.8% in Vf3. Deficits and gains of this magnitude at concentrations approaching the determination limit are characteristic of analytical variability rather than of biological uptake, and the bedding reactors are for that reason not interpreted elsewhere in this work (Table S10).

We tested the interpretation of each component against evidence outside the ordination. PC1 loadings correlate with the measured enrichment of each element between the sludge-bearing and the bedding-only reactors (Spearman $\rho = 0.83$ at day 0 and 0.80 at day 60, $p \leq 0.001$), which identifies it as a provenance axis on data independent of the covariance structure, while PC2 loadings run in the opposite direction against the same quantity at day 0 ($\rho = -0.81$, $p < 0.001$) and are unrelated to it at day 60 ($\rho = -0.37$, $p = 0.19$). PC3 carries titanium alone, for which a rank correlation computed across fourteen elements is uninformative; its interpretation as analytical refractoriness rests on the 60.4% recovery of certified titanium against 96.5% for iron (Section 3.2) and on the absence of a treatment effect on its score. The same test performed against the nominal sludge-derived share calculated from the raw-material analyses does not succeed ($\rho = 0.30$, $p = 0.30$), and the elements responsible are zinc, lead, manganese, and iron, whose nominal baselines are among those that do not reconcile with the measured day-0 values (Section 3.6); the measured enrichment is therefore the appropriate quantity.

The consequence for the argument is direct. The axis carrying the elements charged with the sludge does not separate the two systems, whereas the axis carrying the redox-sensitive elements does. If earthworm processing had removed chromium from the reactors, separation would be expected on PC1, where chromium loads at 0.98. It is not. The ordination therefore locates the treatment effect in the reactor's chemical environment rather than in the chromium inventory, which is the conclusion the mass balance reaches independently.

The elements loading on PC2—manganese, cobalt, zinc, iron, lead, and barium—are those governed by the formation of Mn and Fe oxyhydroxide phases and by sorption and occlusion on them, which, over the measured range of pH 7.35–8.58 under oxidising conditions, is the expected chemistry. That chemistry cannot be isolated from the biology in the present design: substrate pH and the presence of earthworms co-vary completely, and although PC2 scores are related to measured pH ($r = 0.68$, $p = 0.015$), the relation does not survive control for system (partial $r = 0.23$, $p = 0.51$). The composition of PC2 is consistent with redox and pH controls but is not evidence for them independently of the treatment effect, and it is presented here on that basis.

These comparisons are made on the concentration basis and are used for rank order only. Of the thirteen elements in Figure 5, only chromium, vanadium, barium, iron, strontium, and titanium reconcile against their calculated baselines within ±30% (Section 3.6); values for the remainder, aluminium among them, are not quantitatively reliable, and no removal figure derived from them is asserted here. The argument rests on the absence of separation between the two groups, which is a property of the ordering rather than of any individual value, and would be unchanged by moderate error in any one element. Nor is the comparison reversed

when attention is restricted to the elements whose baselines do reconcile: among the eight elements of Figure 4, evaluated on mass-balance recovery rather than on concentration change, mean recovery under vermicomposting is 38.2% for the essential elements (Fe, Mg, and Ca) versus 39.9% for the non-essential (Cr, V, Ba, Sr, and Ti). That subset is too small to support a formal test, but it does not separate either.

Table 6. Apparent removal of thirteen elements, calculated on the conventional concentration basis, grouped by biological essentiality, in each of the sludge-bearing vermicomposting treatments for which day-0 data are available. $n = 13$ elements per treatment. Essential: Cu, Zn, Mn, Fe, Co, and Ni. Non-essential: Cr, Al, Ti, V, Sr, Ba, and Pb. Values are used for group comparison only and are not asserted as quantitative removal (Section "The Deficit Tracks Geochemical Affinity, Not Biological Essentiality"). Element-by-element values are given in Table S14.

Treatment	Essential, Mean (%)	Non-Essential, Mean (%)	Difference (Points)	Mann–Whitney *U*	*p*
E. fetida, 10% sludge	38.9	43.7	−4.8	17.0	0.63
E. eugeniae, 7.5% sludge + biochar	44.3	35.1	+9.2	27.0	0.45
E. eugeniae, 10% sludge	57.3	56.0	+1.3	19.0	0.84

Two-sided exact Mann–Whitney test, $n = 6$ essential and $n = 7$ non-essential elements. With group sizes of this order, the test detects only large separations; the argument rests on the consistency of the null result across three independently prepared reactors and on the inconsistent sign of the difference, not on any single *p* value.

This null result delimits which biological explanation is excluded, and it is a narrow one. What the essentiality test rules out is the retention of chromium in earthworm tissue, the pathway that would require a regulated uptake and storage system; it does not rule out a biological contribution to the deficit, and the data positively require one. The deficit appears only where earthworms are present: the composting control at matched sludge loading recovers 102.7% of the expected chromium, and if earthworm activity were irrelevant, the two systems would not separate. Earthworms therefore act chemically rather than accumulatively and act on the substrate rather than within the animal. Earthworm processing accelerated organic-matter mineralisation, evidenced by dry-matter loss of 13.0–20.0% against 5.7–8.4% under composting, which would release chromium previously complexed by humic and fulvic ligands; it was associated with a greater decline in Cr(VI), to 30.6–45.1% recovery compared with 55.3–67.8% under composting, which mass conservation implies is reduction to Cr(III); and the redistribution observed is consistent with co-precipitation of the released Cr(III) with Fe–Mn oxyhydroxides under the neutral to slightly alkaline, oxidising conditions of the vermireactor, raising the oxide-bound fraction to 72.6–74.4% while the same fraction declined by around 7 percentage points in both composting controls (Section 3.3). The rank order in Figure 5 is consistent with this reading: aluminium, which partitions readily into silicate and oxide phases and has no biological pathway, shows the largest apparent deficit, whereas iron, a constituent of the oxide phases themselves, shows the smallest. The ordering tracks geochemical affinity for the digestion-refractory pool, not biological demand. Bioaccumulation factors below unity in both species should accordingly be read, as set out in Section 3.4, as a favourable outcome rather than a null one.

3.7. Implications for Reported Removal Efficiencies and for Reuse Assessment

The combination of a closed gravimetric mass balance, a partial digestion protocol, and progressive redistribution of chromium into refractory mineral phases provides a plausible alternative explanation for removal efficiencies reported throughout the tannery-sludge vermicomposting literature [8–10,12,18]. Studies that have evaluated the mobile fractions directly report the outcome this reading predicts, a decline in water-soluble and exchangeable chromium [11]. It is important to be clear about what this reinterpretation

does and does not withdraw. What is withdrawn is a claim about mass: chromium was not removed from the reactors and could not have been. What is not withdrawn is the value of earthworm processing, which the same dataset establishes on endpoints unaffected by the digestion issue. Where such studies employ partial digestion, do not report dry-matter loss, and do not constrain their results against a mass balance, an apparent removal of 30–96% is compatible with complete retention of the metal in a progressively less extractable form. The soil-amendment literature distinguishes between an apparent reduction in extractability and a genuine shift of the metal into more stable phases through the choice of extraction scheme, with sequential procedures read as evidence of the latter and single-step extractants of the former [19]. The present data show that the distinction must be carried one step further, to the total determination itself: without a mass constraint, the measured total is also method-dependent, falling by 55–64% here while no chromium left the reactors. The contribution of this work is therefore not another removal figure but a test of what such figures measure, conducted under the one condition in which the question can be settled: a reactor from which the metal cannot leave. Table S8 compares the conventional concentration-basis calculation with the mass-basis calculation for the present dataset and shows that the latter yields apparent removals of 56–65% under vermicomposting, values not physically attainable in a reactor without an export pathway. This does not diminish the value of the treatment: reduction of Cr(VI) and redistribution of chromium towards the Fe–Mn oxide-bound fraction, with the mobile pool held at or below 0.75%, is a substantive and desirable outcome for environmental risk. It does require that the outcome be described as immobilisation and detoxification rather than removal and that vermicompost derived from tannery sludge be evaluated on chromium mobility and speciation rather than on total-chromium reduction. The same asymmetry between endpoints appears in an independent pot study of cadmium-spiked soil amended with vermicompost, biochar, and *Azolla microphylla*, in which the exchangeable fraction fell far more steeply than the total and tissue accumulation in *Eisenia fetida* declined accordingly [22]. The mobile pool is the quantity that responds to treatment, and it is the quantity that carries the risk.

Final chromium concentrations in sludge-bearing treatments remained between 1366 and 1887 mg/kg by partial digestion and, on the mass-balance argument advanced here, the true total is higher still. Application of this material to food-crop systems is not supported. Restricted use in degraded-land rehabilitation, non-food plantation forestry, or sequential treatment remains defensible with respect to chromium on the basis of the low mobile fraction, subject to local regulatory thresholds and to site-specific assessment of long-term redox stability, since Fe–Mn oxide-bound chromium can be remobilised under reducing conditions. Two further pathways warrant attention in a material destined for land application. Manganese oxides are the dominant abiotic oxidant of Cr(III) in soils and can regenerate mobile Cr(VI), at a rate governed by the solubility of the Cr(III)-bearing phase and moderated by Fe(II) availability [45]. Low-molecular-weight organic acids and siderophores, both abundant in composted material and in the rhizosphere, dissolve Cr–Fe (oxy)hydroxides incongruently, preferentially releasing Fe and leaving a Cr-enriched residue of higher predicted solubility [46]. Neither process was evaluated here, and both bear on whether the immobilisation observed at day 60 persists after application to soil where pH, redox fluctuation, and manganese oxides jointly govern the re-formation and transport of Cr(VI) [47]. Suitability for reuse is not, however, established for the material as a whole. As set out in Section 1, a treatment evaluated on total chromium alone cannot establish that the resulting material is suitable for any downstream use, and the co-contaminants that carry independent regulatory significance in this sludge—cadmium and lead in particular—are among those for which the mass balance could not be closed here. Any end-use decision therefore requires a total-recoverable multi-element determination on

the finished material, evaluated against the applicable sludge-application limits, together with a standard leaching test (e.g., TCLP or SPLP) and an assessment of stability under reducing and acidic conditions, which lie outside the scope of the present study.

Three practical consequences follow for managing this waste stream: First, a removal efficiency computed from concentration change is not a safety endpoint for a vermicomposted metal-bearing waste and should not be accepted as one; where it is reported, the accompanying dry-matter loss and the digestion method must be reported with it, since neither the magnitude nor the direction of the apparent change can be interpreted without them. Second, the endpoints that do carry information about human and ecological risks from land application should be reported in its place: hexavalent chromium, the exchangeable and carbonate-bound fraction, a total determination made on a total-recoverable digestion, and a standard leaching test, all of which are measurable by methods already standard in waste-characterisation laboratories, consistent with compost passivation studies, which judge success by the shift of metals from exchangeable and reducible to oxidisable and residual forms rather than by total content [48], so the substitution imposes no new analytical burden. Third, the treatment retains its value under this reading. Destruction of the carcinogenic species, confinement of the residual chromium to a pool at or below 0.75% mobile, and the further gain in Cr(VI) reduction with a locally producible bamboo biochar support a restricted reuse pathway for a waste that currently has none, in a region where chromium-laden tannery effluent still reaches cropland untreated [49]. What the analysis withdraws is a claim about mass; what it leaves is a defensible basis for deciding where this material can and cannot go.

4. Limitations

Total chromium was determined by a single partial acid digestion, applied consistently across all treatments and both sampling times; the comparison between systems is therefore internally valid, but no independent absolute total is available. A total-recoverable or hydrofluoric acid digestion of the same day-60 material remains the principal outstanding requirement. Until it is made, attributing the chromium deficit to incomplete liberation by the partial digestion remains an inference: it is consistent with the reference-material recoveries, the titanium control, and the fractionation data, but it has not been verified directly. The deficit of 55–64% under vermicomposting (Table 3) is therefore reported as unresolved, and the conclusions of this study are confined to what holds whatever its cause: chromium was not exported from the reactors, Cr(VI) declined, and the mobile fraction remained at or below 0.75%.

The mass balance is referenced to a nominal baseline calculated from component analyses because chromium in sub-samples drawn from individual reactors at day 0 varied between 2730 and 5424 mg/kg at nominal identical loading (Table S12a). Recovery was therefore also evaluated against a composite determination made on the combined substrate at reactor filling, which shifts the absolute recoveries upward but preserves the separation between systems (Table S13). Nine elements failed to reconcile between nominal and measured values and are reported without removal-efficiency interpretation (Table S11). Digestions were performed on 0.1 g sub-samples of the ground material; the titanium data indicate that any differential sub-sampling bias between the two systems is an order of magnitude smaller than the chromium deficit (Section "Sub-Sampling and Particle-Size Considerations"), but a contribution to the absolute magnitude of that deficit cannot be excluded.

The mechanism was not resolved: sorption, persistent free radicals, and microbial reduction are named as candidates, none asserted, and the proposed shift to occluded chromium within the Fe–Mn oxide-bound fraction is an interpretation rather than a di-

rect observation. Direct confirmation would require spectroscopic characterisation of the chromium coordination environment, together with the absolute concentrations of the Tessier fractions, neither of which was obtained here. The UV–visible and FT-IR approaches applied to vermicomposted sludge elsewhere [18] indicate oxide-associated forms but are not element-specific. No sterile control was included, so the relative contributions of microbial and abiotic processes to Cr(VI) reduction and to chromium redistribution cannot be separated.

5. Conclusions

In mass-closed reactors with gravimetrically determined dry-matter loss, vermicomposting of chromium-laden tannery sludge with *Eisenia fetida* and *Eudrilus eugeniae* did not remove chromium and could not have done so. Measured concentrations fell to 35.4–44.4% of the mass-balance expectation, while the composting control at matched sludge loading recovered 102.7%, and the exclusion of drainage, tissue retention, and volatilisation leaves a change in the association of chromium that reduces its liberation by partial digestion as the most consistent explanation. The fractionation data and the response of titanium as an internal control support it, but direct confirmation by total decomposition remains outstanding.

The earthworms are nonetheless central to the outcome: the deficit appeared only where they were present. Their role was chemical rather than accumulative, accelerating organic-matter mineralisation, Cr(VI) reduction, and the redistribution of chromium towards Fe–Mn oxyhydroxides, and bioaccumulation factors below unity in both species mean that no secondary contaminant burden was created in worm biomass, consistent with the regulated uptake and excretion kinetics reported for chromium in earthworms [50].

Measured on the endpoints that survive the mass-balance analysis, what the treatment achieved is more useful than what it was previously reported to achieve. Hexavalent chromium, unlike total chromium, can be chemically changed, and recoveries were 30.6–45.1% under vermicomposting versus 55.3–67.8% under composting; biochar-amended vermicomposting achieved both greater Cr(VI) reduction and the only consistent redistribution towards the mineral-associated pool. Residual chromium moved further into the Fe–Mn oxide-bound fraction in three of the four sludge-bearing vermicomposting treatments, reaching 72.6–74.4% of the total, while the readily bioavailable pool stayed at or below 0.75% in the sludge-bearing treatments, and both species behaved as chromium excluders. Vermicomposting therefore detoxifies and immobilises chromium in tannery sludge without removing it. Removal efficiencies derived from partial digestion of vermicomposted material should be interpreted with caution, and mobility- and speciation-based endpoints should be used instead. Direct confirmation of the proposed change in chromium association would require closing the mass balance through total decomposition and solid-phase speciation by synchrotron X-ray absorption spectroscopy, which we identify as the principal next steps.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/environments13110588/s1, Table S1. Reagents and conditions of the Tessier sequential extraction procedure; Table S2a–c. Certified reference material recoveries (LHM, SQC 414, and NCSZC71068 for Cr(VI)); Table S3. pH and total sulphur in compost and vermicompost at day 0 and day 60; Table S4. Cr(VI), Cr(III), and bioaccumulation factors in all treatments at day 0 and day 60; Table S5. Geochemical fractionation of chromium (F1–F5, % of total) at day 0 and day 60; Table S6. Rotated component matrix (Varimax with Kaiser normalisation) with KMO and Bartlett statistics; Figure S1. Heatmap of Varimax-rotated factor loadings before and after treatment; Table S7. Earthworm performance at day 60; Table S8. Apparent chromium removal calculated on a concentration basis and on a mass basis; Table S9. Apparent loss on a

mass basis for the eight elements reconciling within ±30%; Table S10. Gravimetric mass balance for chromium in all twelve reactors; Table S11. Nominal initial concentrations and reconciliation status for all elements, including the nine not reconciling within ±30% and cadmium, which could not be quantified; Table S12a–e. Concentrations of thirteen trace elements and of sodium, magnesium, and calcium in compost and vermicompost at day 0 and day 60; Table S13. Chromium recovery evaluated against the nominal and the measured composite baseline; Table S14. Apparent removal of thirteen elements in each sludge-bearing vermicomposting treatment, grouped by biological essentiality; Table S15. Elemental composition of the bamboo biochar determined by EDS; Table S16. Independent confirmation of the component structure of the day-60 principal component analysis; Figure S2. Chemical properties of the vermicomposting and composting system at day 0 and day 60.

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