



# Vermicompost Amended With Rock Phosphate as a Climate Smart Technology for Production of Organic Swiss Chard (*Beta vulgaris* subsp. *vulgaris*)

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### Specialty section:

This article was submitted to  
Climate-Smart Food Systems,  
a section of the journal  
Frontiers in Sustainable Food Systems

**Received:** 12 August 2021

**Accepted:** 03 January 2022

**Published:** 16 February 2022

### Citation:

Ajibade S, Mupambwa HA,  
Manyevere A and Mnkeni PNS (2022)  
Vermicompost Amended With Rock  
Phosphate as a Climate Smart  
Technology for Production of Organic  
Swiss Chard (*Beta vulgaris* subsp.  
*vulgaris*).  
Front. Sustain. Food Syst. 6:757792.  
doi: 10.3389/fsufs.2022.757792

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Vermicomposting is being promoted as climate-smart agriculture (CSA) technology for developing organic nutrient sources that can be adopted by smallholder farmers. The amendment of soils with cost-effective and available inorganic fertilizers like rock phosphate (RP) and phosphorus-solubilizing microorganisms should also be promoted. Our study investigated the seedling and maturity growth of Swiss chard. The crop maturity study investigated the effect of amending an Oxisol soil with three phosphorus (P) sources applied at three different rates (0, 25, and 50 mg P kg<sup>-1</sup> soil), cow and pig manures, and P-solubilizing bacteria resulting in the following treatments: control (soil), soil + RP alone, soil + RP-enriched cow manure vermicompost (VC) with phosphorus-solubilizing bacteria (PSB), and soil + RP-enriched pig manure vermicompost (VP) with PSB. The study investigated the growth performance and accumulation of copper (Cu), zinc (Zn), chromium (Cr), and lead (Pb) in the edible parts of Swiss chard (*Beta vulgaris* subsp. *vulgaris* var. *Cicla*). The seedlings were grown using pine bark compost and the three P sources (VC, VP, and RP) at five different application rates (0, 25, 50, 75, and 100%). The results revealed that the use of 50 mg P kg<sup>-1</sup> as VP gave the highest fresh weight of 39.78 g, leaf area of 240.41 cm<sup>2</sup>, and a total P in tissues of 326.91 mg kg<sup>-1</sup> at 8 weeks after transplanting. The 50 mg P kg<sup>-1</sup> application as VC resulted in a higher Zn content of 8.50 mg kg<sup>-1</sup>, which was above the permissible level. These results suggest that the best treatment mixture for establishing fully matured Swiss chard was the 50 mg P kg<sup>-1</sup> as VP. Therefore, pig manure vermicompost that has been inoculated with phosphorus-solubilizing microorganisms is a promising CSA technology that can improve organic vegetable production by smallholder farmers.

**Keywords:** organic P amendment, microbial inoculant, acidic soil, heavy metal accumulation, nutrient content

## INTRODUCTION

The negative effect of global climate change in the agricultural sector will mostly affect Sub-Saharan Africa (Antwi-Agyei et al., 2014; Müller et al., 2014). Over 25% of soils in Africa are acidic, and most of these occur in the wetter parts of the continent (FAO and ITPS, 2015). In South Africa, about 5 million ha of soils are severely acidified, with an additional 11 million ha of moderately acidified soil (Venter et al., 2001). Most of these soils are deficient in P and need supplementation with phosphate fertilizers for optimum plant growth (Zaharah et al., 2014; Ch'ng et al., 2019). However, inorganic phosphate fertilizers tend to be adsorbed when applied to acidic soils due to highly reactive  $Al^{3+}$  and  $Fe^{3+}$  cations in these soils, which reduces their agronomic effectiveness (Adnan et al., 2003). Also, long-term phosphate fertilizer's applications result in heavy metal soil concentrations, so their application may lead to heavy metal accumulations in soils (Cheraghi et al., 2012). For instance, the soil application of synthetic phosphate fertilizers at a rate of as low as 20 kg P  $ha^{-1}$  resulted in the accumulation of elements such as Cr and Hg (Kongshaug et al., 1992; Kpomblekou and Tabatabai, 1994).

Rock phosphate (RP) is encouraged, especially in organic farming, but its effectiveness as a P source is highly dependent on its solubility in the soil (Mupambwa and Mnkeni, 2018). For example, the igneous RP found in South Africa is low grade with low reactivity and solubility, resulting in low bioavailability of P in the soil (Mineral Information Institute, 2003). Furthermore, the low-grade RP found in South Africa contains chemical properties such as CaO (54.60%), MgO (0.26%),  $P_2O_5$  (40.30%), chromium (18.04 mg  $kg^{-1}$ ), cadmium (1.20 mg  $kg^{-1}$ ), lead (6.05 mg  $kg^{-1}$ ), copper (5.85 mg  $kg^{-1}$ ), and zinc (13.22 mg  $kg^{-1}$ ).

The low bioavailability of RP could be enhanced through co-application with cost-effective and environmentally friendly biofertilizers with high P-solubilizing capacity. There is growing evidence that some microorganisms can enhance the solubilization of RP. More recently, a study by Ajibade et al. (2020) demonstrated the capacity of *Pseudomonas fluorescens* to solubilize a low-grade RP during the cow and pig manure RP-enriched vermicomposting process. The highest Olsen P content of 3,342.64 and 3,051.20 mg  $kg^{-1}$  after week 6 of vermicomposting was observed in the cow and pig manure RP-enriched vermicompost treatments inoculated with *P. fluorescens*. Panda et al. (2013) also concluded that low-grade RP and *P. fluorescens* could help improve P availability and mobilization in P-deficient soils for crop production. The vermicomposting of RP-enriched organic materials has also been found to enhance P release from RP (Unuofin and Mnkeni, 2014; Mupondi et al., 2018).

de Souza et al. (2013) showed that, in addition to P release, the bioavailability of potentially phytotoxic elements like Al, Cr, Ba, Cu, Co, Mn, Pb, Fe, and Zn from RP is also enhanced during vermicomposting. Once released, some of these elements can be taken up by plants and settle in the edible parts of the plants, posing a potential health hazard to consumers. Organic matter has been shown to bind micronutrients and heavy metals and influence their speciation in the soil (Dhaliwal et al., 2019). Likewise, the use of earthworms during vermicomposting is

reported to reduce the resulting vermicompost's heavy metal concentration and toxicity (He et al., 2016; Mupondi et al., 2018). Mupondi et al. (2018) investigated the effect of applying different rates (2, 4, and 8%) of RP during cow manure-waste paper vermicomposting on micronutrient and heavy metal release. They found that during the early stages of vermicomposting, the incorporation of RP resulted in an increased micronutrient and heavy metal content, after which it decreased by 45, 35, 40, 40, and 35% for Zn, Cu, Pb, Cr, and Cd, respectively, after 8 weeks of vermicomposting. This effect was attributed to metal bioaccumulation by the earthworms.

There are few reported studies that have evaluated the effectiveness of RP-enriched vermicompost inoculated with phosphorus-solubilizing microorganisms as a source of nutrients for leafy vegetables grown in acid soils. There is also a lack of information regarding RP-enriched vermicompost and the direct application of RP in acid soils on plant growth, nutrient content, and metal accumulation in edible leafy vegetables such as Swiss chard, cabbage, kale, and lettuce.

The objectives of this study were to investigate the effects of RP alone and in combination with animal manure-waste paper vermicompost on the

- (i) Resulting substrate chemical properties and Swiss chard (*Beta vulgaris* subsp. *vulgaris* var. *Cicla*) seedling growth, and
- (ii) Growth and heavy metal (copper, zinc, cadmium, and lead) uptake by Swiss chard (*B. vulgaris* subsp. *vulgaris* var. *Cicla*).

## MATERIALS AND METHODS

The experiment was conducted in two stages. Stage 1 was a seedling growth experiment, while stage 2 was a Swiss chard maturity experiment. The Swiss chard seeds were first planted in three different organic mixtures (i.e., cow manure RP-enriched vermicompost + pine bark compost, pig manure RP-enriched vermicompost + pine bark compost, and RP alone + pine bark compost) to maintain uniform, healthy, and vigorous seedlings ready to transplant into the mixture of organic amendments and Oxisol soil for the crop maturity stage of the study.

### Stage 1: Seedling Growth Study Source of Growing Material

The RP-enriched cow and pig manure vermicompost with phosphorus-solubilizing bacteria (PSB) (*P. fluorescens*) prepared as described by Ajibade et al. (2020) was used for the seedling stage. The growth media used for the seedling study consisted of commercial pine bark compost that was collected from Rance Timbers, Stutterheim, Eastern Cape, South Africa. The pine bark compost was selected in the present study due to its limited buffering capacity (Mupambwa et al., 2017a) and low chemical and physical properties (Mupondi et al., 2014; Mupambwa et al., 2017b), including its low micronutrient content (Table 1), to show the effect of substituting pine bark compost with nutrient-rich organic fertilizers on improved chemical properties of pine bark for plant growth. The pine bark compost, RP-enriched cow, and pig manure vermicompost were initially

**TABLE 1** | Macro and micro-nutrient content of rock phosphate (RP) cow and pig manure vermicompost and soil type.

Chemical properties	RP-enriched vermicompost type			Soil type
	PB compost	VC + PSB	VP + PSB	Oxisol
pH (H <sub>2</sub> O)	3.80 ± 0.03	8.99 ± 0.14	7.65 ± 0.09	4.07 ± 0.09
EC (mS/cm)	7.60 ± 1.85	1.68 ± 0.19	1.79 ± 0.14	4.65 ± 0.3
Extractable NH <sub>4</sub> -N (mg kg <sup>-1</sup> )	211.95 ± 6.93	292.63 ± 5.60	318.44 ± 16.086	30.40 ± 3.83
Extractable NO <sub>3</sub> -N (mg kg <sup>-1</sup> )	42.20 ± 2.75	118.65 ± 12.11	121.10 ± 16.13	33.94 ± 3.67
Extractable P (mg kg <sup>-1</sup> )	412.40 ± 48.11	1,150 ± 103.85	1,350 ± 219.23	2.3 ± 0.11
<b>Macro-nutrients (mg kg<sup>-1</sup>)</b>				
Ca	3,784.4 ± 157.72	4,712.39 ± 204.77	4,473.24 ± 197.22	221.00 ± 0.00
Mg	431.66 ± 12.32	555.35 ± 44.78	619.19 ± 49.19	39.94 ± 2.47
Na	54.56 ± 7.57	1,019.33 ± 40.10	601.93 ± 40.34	42.02 ± 2.14
K	550.8 ± 17.60	4,390.06 ± 135.16	2,017.62 ± 99.83	129.04 ± 12.87
<b>Micro-nutrients (mg kg<sup>-1</sup>)</b>				
Cu	Bdl	15.79 ± 5.60	4.54 ± 1.61	5.03 ± 0.17
Zn	Bdl	52.45 ± 18.50	17.49 ± 6.18	2.38 ± 0.08
Cr	Bdl	0.04 ± 0.00	0.04 ± 0.01	0.03 ± 0.12
Pb	Bdl	0.052 ± 329.90	0.57 ± 0.20	4.12 ± 0.17

Analysis of soil and RP cow and pig manure vermicompost before the start of the experiment.

Parameters reported as mean ± standard deviation (n = 3).

bdl, below detectable limits; PB, pine bark; VC, cow manure vermicompost; VP, pig manure vermicompost; PSB, phosphorus-solubilizing bacteria.

air-dried and characterized for the various chemical properties (Table 1).

### Treatments, Experimental Design, and Trial Establishment for Seedling Stage

The test crop used in the study was Swiss chard (*B. vulgaris* subsp. var. *Cicla*). It was chosen because it is widely consumed in Sub-Saharan Africa (Mavengahama, 2013) but with high heavy metal bioaccumulation from soil (Römer and Keller, 2001). The seedling part of the experiment was carried out in a glasshouse with temperature and humidity (22°C and 72%, respectively), controls at the University of Fort Hare, Alice campus (32° 46' 59.99" S, 26° 52' 59.99" E). The experimental layout was a factorial design with two factors, which are manure type (i.e., RP-enriched cow vermicompost or RP-enriched pig manure vermicompost) and pine bark compost. The second factor was the substitution level of vermicompost to pine bark compost, i.e., 0, 25, 50, 75, and 100% (v/v). These factors gave a 3 × 5 treatment structure, replicated three times. Polystyrene trays with 30 cavities each were filled with different media combinations of RP-enriched vermicompost and pine bark compost according to the nine treatments. Each treatment was replicated three times for a total of 27 trays. The 27 trays were then laid out in a completely randomized design in the glasshouse. Two Swiss chard seeds were sown in each cavity.

### Seedling Study Measurements

At week 1 after planting, the germination percentage was determined by counting the number of cavities with germinated seeds, along with the plant height. At week 4 after germination, the plant height was recorded and measured from the base of the plant to the tip of the open leaf and was expressed in centimeters,

along with chlorophyll content, which was determined using a SPAD meter (Konica Minolta SPAD meter-520 Plus).

### Stage 2: Crop Maturity Study Source of Growing Material

The sampled Ferralitic soils (IUSS Working Group WRB, 2015) are highly weathered dystrophic belonging to the Oxisol soil order as described in the Soil science division staff, soil survey manual (Ditzler et al., 2017) collected from the Hogsback mountains (32° 33.673' S, 26° 54.607' E), Eastern Cape Province of South Africa. The soils have a high acid saturation (50%) and low pH (KCl) of 4, dominated by kaolinites, hematite, goethite, Fe and Al oxides, and gibbsite clay minerals. The Oxisols were generally the most stable and recommended as prime cropping land that should be optimized for crop production (Manyevere et al., 2016). Initially, the soil was air-dried and characterized for various chemical properties (Table 1). The RP-enriched cow and pig manure-waste paper vermicompost multiplied were used as a P source and amendment for Oxisol soil with unprocessed igneous RP acting as a control.

### Treatments, Experimental Design, and Trial Establishment Maturity Stage

The Swiss chard seedlings used were transplanted from the seedling stage of the experiment and were selected from the growing medium with 50% pine bark compost and 50% RP pig manure vermicompost. The experimental layout was a factorial design with two factors, i.e., phosphorus source (RP-enriched cow manure vermicompost, RP-enriched pig manure vermicompost, and rock phosphate as a control) and P level (0, 25, and 50 mg kg<sup>-1</sup>). The P amendments were incorporated into the Oxisol soil to supply each 25 mg kg<sup>-1</sup> P and 50 mg kg<sup>-1</sup> P

(Bray 1). The amount of RP vermicompost required to amend the acid soil to reach the target P levels was determined using Equation 1.

$$V = \frac{P_{req}}{P_{verm}} \times 1 \text{ kg RP vermicompost} \quad (1)$$

where  $V$  is the vermicompost added to reach the target P level per kilogram soil, and  $P_{req}$  (mg) is P content required to reach target P, while  $P_{verm}$  (mg) is the extractable P in the vermicompost.

Two seedlings were placed in plastic pots of 2.5-L capacity filled with 2 kg of Oxisol soil and incorporated with P amendments from the six treatment combinations, which were replicated three times. The pots were kept in a glasshouse with temperature and humidity controls (22°C and 72%, respectively). When required, watering was done using tap water with no external fertilizer being applied until maturity.

### Maturity Study Measurements

The Swiss chard was harvested 2 months after planting, and during this time, the fresh Swiss chard weight, Swiss chard height (distance from medium potting level to the top of the leaf), leaf area (using leaf area meter) were measured, and chlorophyll content was determined as in the seedling part of the study. The shoots were oven-dried at 60°C, and the dried shoots were ground (<1 mm) and analyzed for tissue N, P, and K and trace elements, i.e., Cu, Zn, Cr, and Pb by wet digestion using a mixture of concentrated sulfuric acid, selenium, salicylic acid, and hydrogen peroxide as described by Okalebo et al. (2002). The extract was filtered and then analyzed using ICP-OES (Varian Inc., The Netherlands), and tissue N was analyzed using a UV/Vis spectrophotometer (Helios Gamma, Thermo Spectronic, Cambridge, United Kingdom).

### Chemical Properties of the Growing Media pH and Electrical Conductivity

The pH and the electrical conductivity (EC) were determined in the water at the ratio of 1:10 ( $w/v$ ) using a pH meter equipped with a glass electrode (Crison Basic 20+, Crison Instruments SA, Barcelona, Spain) (Yan et al., 2016).

### Olsen P

A solution of 0.5 M sodium hydrogen carbonate, adjusted to a pH of 8.5 using 1 M sodium hydroxide, was used for extraction. A 2.5-g sample of compost was extracted with 50 ml of the 0.5 M  $\text{NaHCO}_3$  solution and was shaken for 30 min at 180 rpm using a horizontal shaker. After shaking, samples were filtered through Whatman™ No. 42 filter paper, and the filtrates were then adjusted to a pH of 5 using 2.5 ml of sulfuric acid before they were analyzed. For the analysis of P in the solution, the colorimetric ascorbic acid method was used (Agric Laboratory Association of Southern Africa AGRILASA, 2004).

### Bray-1

Extractable Bray-1 P was determined by extracting 6.67 g of sample in 50-ml Bray-1 solution. The contents of the mixture were shaken manually for 1 min, filtered, and analyzed for extractable Bray-1 P. The amount of orthophosphate extracted

was determined using a UV/Vis spectrophotometer (Helios Gamma, Thermo Spectronic, Cambridge, United Kingdom) employing the ascorbic acid method, as described by Agric Laboratory Association of Southern Africa AGRILASA (2004).

### Extractable Ammonium and Nitrate and Nitrite

Inorganic nitrogen ( $\text{NH}_4\text{-N}$ ,  $\text{NO}_3\text{-N}$ , and  $\text{NO}_2\text{-N}$ ) were extracted using 0.5 M potassium sulfate ( $\text{K}_2\text{SO}_4$ ) at a ratio of 1:10 ( $w/v$ ). Then, 10 g of wet homogenous sample was weighed and mixed with 100 ml of 0.5 M  $\text{K}_2\text{SO}_4$  into a 150-ml plastic bottle and shaken on a reciprocating shaker for 60 min at 180 rpm. The suspension was then filtered using Whatman™ No. 42 filter paper (Maynard et al., 2006). The ammonium concentration was determined based on the Berthelot reaction involving salicylate, while the concentration of nitrate and nitrite was determined based on the cadmium reduction method, all done using a UV/Vis spectrophotometer (Helios Gamma, Thermo Spectronic, Cambridge, United Kingdom).

### Exchangeable Cations

Mg, Ca, K, and Na were extracted using 50 ml of ammonium acetate (1:10  $w/v$ ). Then, 5 g of air-dried samples were weighed, placed in a 10-ml extraction bottle, and shaken horizontally in a reciprocating mechanical shaker for 30 min at 180 rpm. The extract was filtered and analyzed using ICP-OES (Varian Inc., The Netherlands).

### Trace Elements

The plant available metal elements Cu, Zn, Cr, and Pb were determined using diethylenetriaminepentaacetic acid-triethanolamine (DTPA-TEA) extract as described by Reed and Martens (1996). The extractant consisted of 0.005 M DTPA, 0.1 M TEA, and 0.01 M  $\text{CaCl}_2$  with a pH adjusted to 7.3. The plant test involved shaking 10 g of dry sample with 20 ml of the DTPA extractant on a reciprocating mechanical shaker for 2 h at 180 rpm. The sample extract was filtered using Whatman™ No. 2 filter paper, and the plant available metal elements (i.e., Cu, Zn, Cr, and Pb) in the filtrate were analyzed using ICP-OES (Varian Inc., The Netherlands).

### Data Analysis

The two studies were all factorial designs, and factorial analysis of variance was done for the data using JMP version 14.0 statistical software (SAS Institute, Inc., Cary, North Carolina, U.S.A.). Microsoft Excel 2010 (Microsoft, Washington, U.S.A.) was used to construct all graphs.

## RESULTS

### Seedling Development in Pine Bark Substituted With Rock Phosphate-Amended Cow and Pig Manure Vermicompost—Stage 1

#### Planting Media pH and Electrical Conductivity

There was a significant ( $p < 0.001$ ) difference in the pH and EC of the planting media between different substitution rates of pine bark compost and vermicompost type (**Supplementary Table 1**).

In all the treatment combinations, regardless of vermicompost type, i.e., pig or cow, the pH increased with an increase in the amendment level of vermicompost, with 100% vermicompost having the highest pH of 8.80 for cow manure vermicompost and 7.76 for the pig manure vermicompost (**Supplementary Figure 1A**). The lowest pH was observed in the 100% pine bark, with a pH of 3.77. A trend to pH was also observed on EC, with the higher the level of vermicompost substitution having the highest EC (**Supplementary Figure 1B**). Interestingly, the EC was significantly higher for the cow manure vermicompost than the pig manure vermicompost. For 100% cow manure or pig manure vermicompost, the EC was observed to be 19.03 and 11.07 mS/cm, respectively, while for control treatments, it was 7.60 mS/cm.

### Seed Germination

The vermicompost type and the level of substitution into pine bark compost showed highly significant ( $p < 0.001$ ) effects on the germination of Swiss chard seedlings at week 1 after planting (**Supplementary Table 1**). However, there was no consistent trend on the effects of different substitution levels on seed germination. Only the control had the lowest germination after 1 week of planting, while wide variations were observed between the 25 and 100% substitution level (**Supplementary Figure 2**). This highest germination was observed in the treatment with 50%-enriched pig manure vermicompost, with a germination percentage of 96.67%, while the substitution with 25% cow manure vermicompost gave a germination percentage of 91.67%. Compared to the control treatment (pine bark compost) with a germination percentage of 20%, the control treatment was 71.3% lower than the highest germination percentage of the treatment with 50% RP-enriched pig manure vermicompost.

### Seedling Development

The substitution of RP-enriched cow manure and pig manure vermicompost into pine bark compost showed a highly significant ( $p < 0.001$ ) influence on the plant height of Swiss chard seedlings at week 4 after germination (**Supplementary Table 1**). The pig manure vermicompost across all the treatments resulted in the highest plant height compared to the cow manure-based treatments (**Supplementary Figure 3**). The substitution of pine bark with 50% pig manure vermicompost gave the highest seedling height of 6.33 cm, while the lowest was also observed at the 50% cow manure vermicompost with a seedling height of 2.44 cm. On the other side, the substitution of RP-enriched cow manure vermicompost at 75% gave the highest seedling height of 4.28 cm. The seedling height in the treatment with 50% pig manure vermicompost was 33.50% higher than that in the control treatment. The cow manure vermicompost had a percentage difference of 1.68% in the treatment with 75% cow manure vermicompost.

There was a significant ( $p < 0.001$ ) difference in the chlorophyll content between the control treatment and the various RP vermicompost-substituted treatment mixtures at week 4 after germination (**Supplementary Table 1**). Among the different vermicompost types, the highest chlorophyll content

**TABLE 2 |** ANOVA analysis for Swiss chard grown in RP-enriched cow and pig manure vermicompost with Oxisol soil at week 8 after transplanting.

Source variation	Fresh weight (g)	Plant height (cm)	Leaf area (cm <sup>2</sup> )	SPAD
Phosphorus source	<0.001	<0.001	<0.001	<0.001
Phosphorus level	<0.001	<0.001	<0.001	0.0226
P level × P source	<0.001	<0.001	<0.001	<0.001

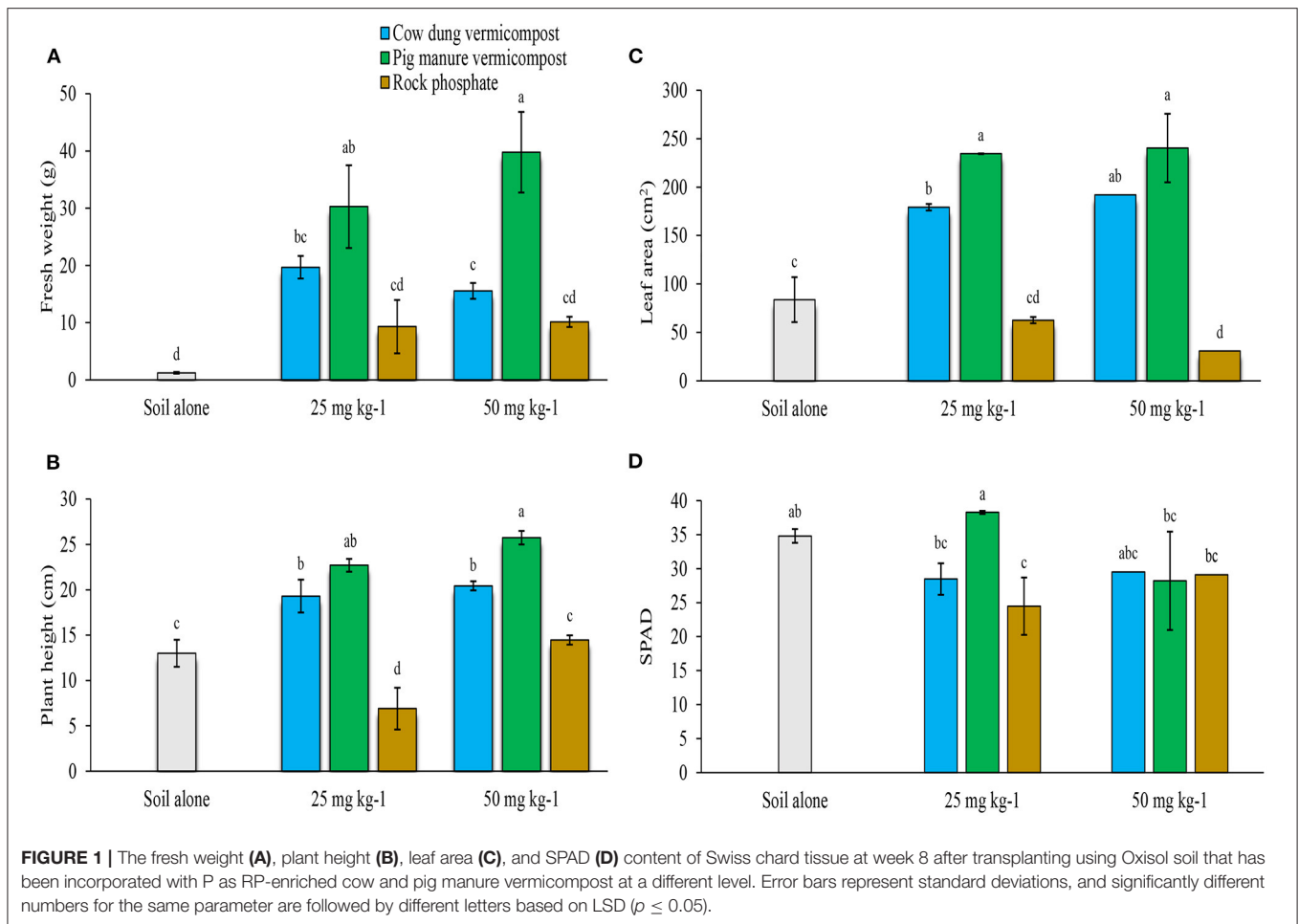
was observed in the treatment with pig manure vermicompost (**Supplementary Figure 4**). The highest chlorophyll content of 30.48 was observed in the 75% pig manure-substituted treatment, while for the cow manure vermicompost, this was observed at 100% substitution. The chlorophyll content of the seedling in the control treatment was 67.20% lower than that in the treatment with 75% pig manure vermicompost. The chlorophyll content in the control treatment was 51.70% lower than that in the treatment with 100% cow manure vermicompost.

## Seedling Maturity in Soil Substituted With Rock Phosphate-Amended Cow and Pig Manure Vermicompost—Stage 2 Swiss Chard Yield Parameters

In the Swiss chard maturity part of the study, two vermicomposts were used as sources of phosphorus supplying either 25 or 50 mg kg<sup>-1</sup> of phosphorus and a soil-alone control. The P source and P level had a significant ( $p < 0.001$ ) influence on the fresh weight of the Swiss chard plant at week 8 after transplanting (**Table 2**). Pig manure vermicompost had the highest weight of 39.80 g, followed by the cow manure vermicompost, RP alone, and the soil alone (**Figure 1A**). The fresh weight increased as the P level increased for the pig manure vermicompost, though this was not the case for other treatments.

The plant height of the Swiss chard at three different P levels is presented in **Figure 1B**. The analysis of variance showed that the Swiss chard plant height was significantly ( $p < 0.001$ ) affected by P source and different levels of P. It was noticed that the application of RP-enriched pig manure vermicompost at an application rate of 50 mg P kg<sup>-1</sup> recorded higher plant height of 25.75 cm, followed by the application rate of 25 mg P kg<sup>-1</sup> with a plant height of 22.70 cm over the use of RP alone and control treatment. The percentage difference in plant height between the treatment with 50 mg P kg<sup>-1</sup> as pig manure and the control treatment was 49.51%.

The P source and P level had a significant ( $p < 0.001$ ) influence on the leaf area of the Swiss chard plant at week 8 after transplanting (**Table 2**). The Swiss chard plant fertilized with RP-enriched pig manure vermicompost at an application rate of 50 mg P kg<sup>-1</sup> resulted in a maximum leaf area of 240.41 cm<sup>2</sup>. Compared to the leaf area content observed in the control treatment, the percentage difference of higher than 65.16% was observed after applying 50 mg P kg<sup>-1</sup> as pig manure



vermicompost (Figure 1C). However, the application of RP alone, respectively, gave a minimum leaf area of 62.57 and 30.65 at a rate of 25 and 50 mg P kg<sup>-1</sup>, which was 25.30% and 63.41% lower than the control treatment, respectively.

A highly significant ( $p < 0.001$ ) influence of P source in the chlorophyll content of Swiss chard tissue was observed. Furthermore, the level of P had a significant ( $p < 0.05$ ) influence on the chlorophyll content of Swiss chard tissue (Table 2). An application rate of 25 mg P kg<sup>-1</sup> pig manure vermicompost showed the highest chlorophyll content of 38.28, which was 9.08% higher than the control treatment (Figure 1D). The minimum chlorophyll content was observed in the treatment with 25 mg P kg<sup>-1</sup> RP alone, with a chlorophyll content of 24.47, 29.70% lower than the control treatment. Therefore, the application of pig manure vermicompost at an application rate of 25 mg P kg<sup>-1</sup> showed increased greenness of Swiss chard tissue, as the chlorophyll content was high compared to other treatments.

### Mature Swiss Chard N, P, and K Contents

A significant ( $p < 0.05$ ) effect of P source and different P levels (0, 25, and 50 mg kg<sup>-1</sup>) on the total N of Swiss chard tissue was observed (Table 3), with a highly significant ( $p < 0.001$ ) effect

of P source and P level on total K of Swiss chard tissue. The application of RP alone at 25 and 50 mg P kg<sup>-1</sup> gave the highest total N tissue content of 4.95 and 4.99%, respectively, which was 13.81 and 14.53% higher than the control treatment (Figure 2A). However, the total N tissue content among the different treatment combinations did not show significant variations since the lowest total N tissue content of 4.72% was recorded in the 50 mg P kg<sup>-1</sup> pig manure vermicompost treatment. The highest application rate of 50 mg P kg<sup>-1</sup> showed the highest tissue P content compared to the application rate of 25 mg P kg<sup>-1</sup>. However, the lower application rate of 25 mg P kg<sup>-1</sup> as RP alone resulted in the highest tissue P content of 327.30 mg kg<sup>-1</sup> (Figure 2B), which was 39.45% higher than the control treatment. After the control treatment, the minimum P tissue content was observed in the treatment with 25 mg P kg<sup>-1</sup> cow manure vermicompost. RP-enriched vermicompost and RP alone in both the application rates of 25 and 50 mg P kg<sup>-1</sup> gave a similar trend in tissue K content excluding the control treatment and was in the order RP-enriched cow manure vermicompost > RP-enriched pig manure vermicompost > RP alone. Moreover, the highest tissue K content was observed in the RP-enriched cow manure vermicompost treatment at an application rate of 50 mg P kg<sup>-1</sup> (Figure 2C).

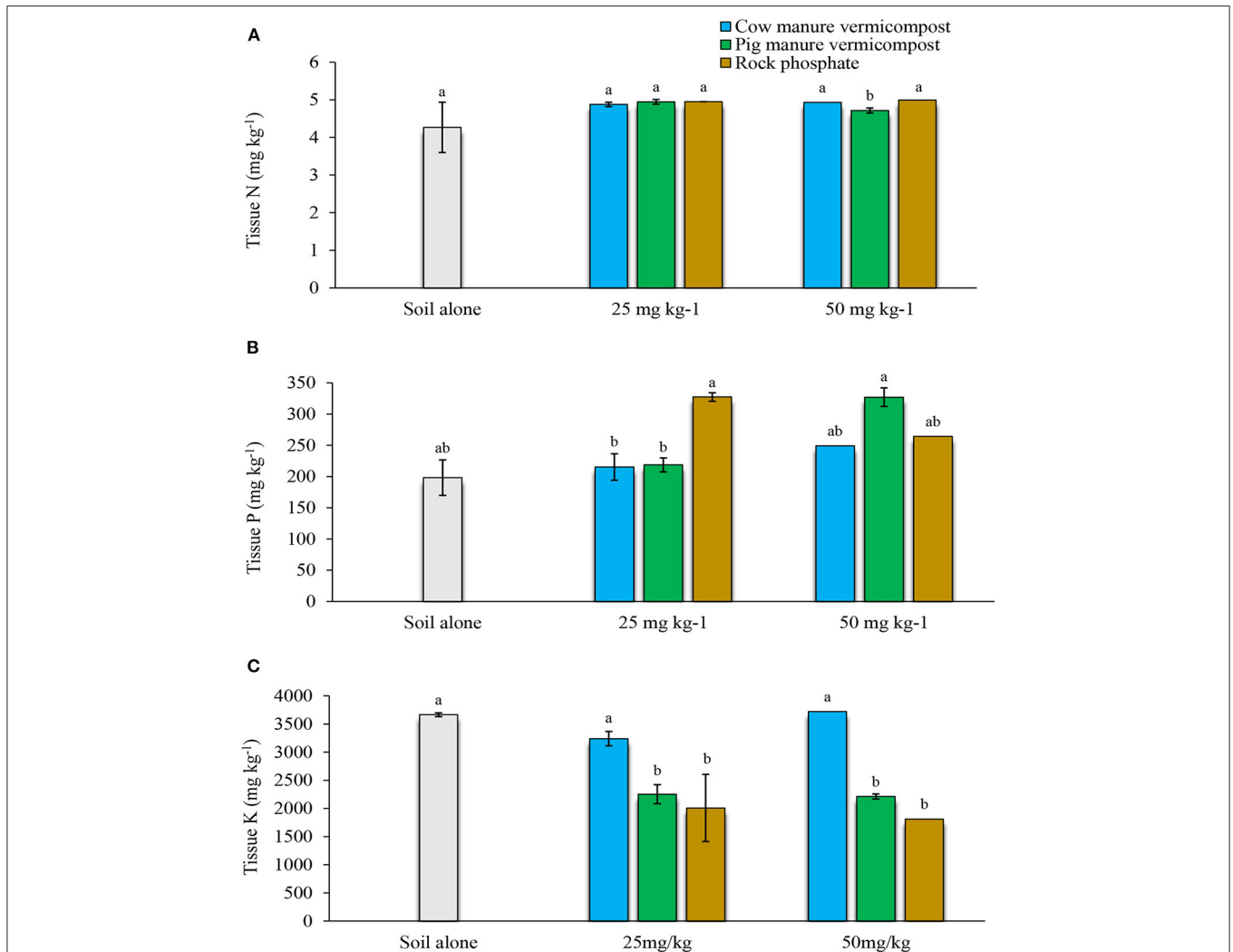
### Swiss Chard Tissue Heavy Metal (Cu, Zn, Cr, and Pb) Concentrations

The effect of P source and P level showed a significant ( $p < 0.05$ ) influence on the amount of Cu, Zn, Cr, and Pb of Swiss chard tissue planted in Oxisol soil (Table 3). The Cu content in all the treatment combinations ranged from 0.42 to 0.64 mg kg<sup>-1</sup>, with the highest Cu content on the treatment with 50 mg P kg<sup>-1</sup> cow manure vermicompost and the lowest

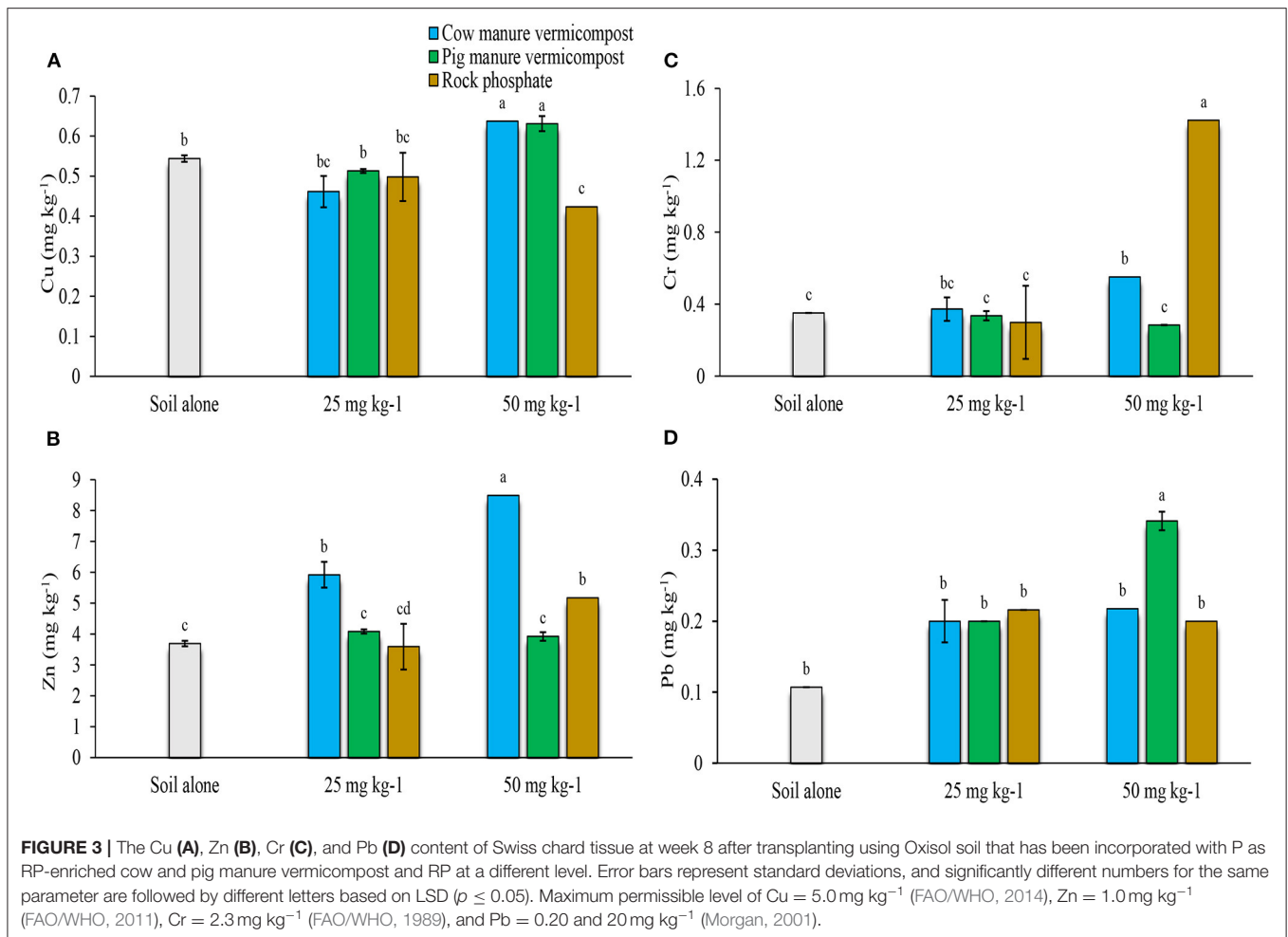
concentration on the treatment with 50 mg P kg<sup>-1</sup> as RP alone (0.42 mg kg<sup>-1</sup>) (Figure 3A). Regarding Zn in Swiss chard tissue, the highest content was recorded on the treatment with 50 mg kg<sup>-1</sup>, followed by 25 mg P kg<sup>-1</sup> cow manure vermicompost with Zn content of 8.50 and 5.92 mg kg<sup>-1</sup>. Compared to the control treatment, an increase of 56.60 and 37.68% was observed after 50 and 25 mg P kg<sup>-1</sup> cow manure vermicompost (Figure 3B).

**TABLE 3 |** ANOVA for Swiss chard chemical properties in RP-enriched cow and pig manure vermicompost with Oxisol soil at week 8 after transplanting.

Source variation	N (%)	P	K	Cu	Zn	Cr	Pb
				mg kg <sup>-1</sup>			
Phosphorus source	0.0022	ns	<0.001	0.0003	<0.001	<0.001	<0.001
Phosphorus level	0.0066	ns	<0.001	0.0002	<0.001	<0.001	<0.001
P level × P source	<0.001	0.0056	<0.001	<0.001	<0.001	<0.001	<0.001



**FIGURE 2 |** Total N (A), P (B), and K (C) content of Swiss chard tissue at week 8 after transplanting using Oxisol soil that has been incorporated with P as RP-enriched cow manure and pig manure vermicompost and RP at a different level. Error bars represent standard deviations, and significantly different numbers for the same parameter are followed by different letters based on LSD ( $p \leq 0.05$ ).



At the P level of 50 mg P kg<sup>-1</sup>, the highest Cr content was found after applying RP alone, with a Cr content of 1.42 mg kg<sup>-1</sup>. Moreover, this highest Cr content was followed by the cow manure vermicompost treatment at the same P level of 50 mg kg<sup>-1</sup> with a Cr content of 0.55 mg kg<sup>-1</sup> (Figure 3C). On the other hand, the Pb content of Swiss chard tissue in all the treatment combinations was <0.4 mg kg<sup>-1</sup>, with the highest Pb content of 0.34 mg kg<sup>-1</sup> in the treatment where a P level of 50 mg P kg<sup>-1</sup> was applied (Figure 3D). Compared to the treatment with 25 mg P kg<sup>-1</sup> as pig manure vermicompost, the Pb content in the treatment was 41.35% lower than the treatment with 50 mg P kg<sup>-1</sup> as pig manure vermicompost and 68.62% lower than the control treatment. Lastly, the data on post-harvest soil chemical properties as influenced by cow manure and pig manure RP vermicompost is given in **Supplementary Table 2**.

## DISCUSSION

### Effect of RP-Enriched Cow and Pig Manure Vermicompost on the Swiss Chard Seedling Growth

The highest growth was observed for the seedling germination rate when the RP-enriched pig manure vermicompost (VP)

application rate was increased from 0 to 50%. The germination percentage was more than 90% at this application rate, and based on Mupondi et al. (2014), the emergence percentage of 90% and above is considered satisfactory for composts. The lowest germination percentage was observed in the control treatment with 100% pine bark compost, possibly due to its acidic nature. Therefore, it is better to increase the pH of pine bark compost by applying 50% pig manure RP vermicompost since the Swiss chard is sensitive to acidic conditions and requires a neutral pH of between 6.0 and 6.8 for its optimum growth. The lowest RP-enriched pig manure vermicompost application during the seedling stages resulted in the highest plant height. These findings are in line with the results of Atiyeh et al. (2001), who observed enhanced growth and yield of tomato seedlings after the application of 50%:50% of pig manure vermicompost and standard commercial greenhouse container medium (Metro-Mix 360), compared to seedling grown using 100% pig manure vermicompost. Mupambwa et al. (2017a) observed that the addition of fly ash-enriched cow manure-waste paper vermicompost to pine bark compost at a ratio of 50%:50% significantly transformed the physical properties of pine bark compost by creating more ideal conditions for the effective emergence of an ornamental marigold plant. They also



observed a percentage difference of 33.7 and 29.23% in the medium with 25% vermicompost compared to the application rate of 75 and 50%. The observed data show a balanced nutrient composition through the application of 50%:50% and 25%:75% RP-enriched pig manure vermicompost into pine bark compost. These findings could be explained by the presence of plant growth regulators like gibberellins, auxins, and kinetins produced during vermicomposting (Kiyasudeen et al., 2015).

During week 4 after germination, the highest chlorophyll content observed in the treatment with 75% pig manure vermicompost could be explained by the high Mg content observed in the pig manure vermicompost with pine bark compost shown (Table 1). This is because the chlorophyll content formation in leaves is related to the availability of physiologically active Mg in plant-available form. The RP-enriched pig manure vermicompost gave the highest tissue chlorophyll content between the vermicompost types compared to the RP-enriched cow manure vermicompost. This might be associated with the availability of essential nutrients for Swiss chard growth in RP pig manure vermicompost, like nitrogen, which is highly required for Swiss chard growth. For instance, Ling et al. (2014) stated that the chlorophyll content of leaf tissue is one of the most important parameters that is measured as the indicator of leaf nitrogen content, chloroplast development, general plant health, and photosynthetic capacity. It was observed from the results that the application of 50 mg P kg<sup>-1</sup> as RP-enriched pig manure vermicompost in Oxisol soil recorded the highest fresh weight, plant height, and leaf area, followed by the application rate of 25 mg P kg<sup>-1</sup>, which showed the highest chlorophyll content.

### Effect of RP-Enriched Cow and Pig Manure Vermicompost on the Growth and Nutrient Content of Swiss Chard Tissue

Even though the direct application of RP in soils with pH 5.5 or less is a low-cost option for P fertilization in soils (International Fertilizer Industry Association IFA, 2013), the direct application of RP in Ferrasol soil used in the study (pH = 4.07) is not good for the growth of Swiss chard in terms of fresh weight, plant height, and leaf area (Figures 1A–C). The findings could be explained by Al<sup>3+</sup> presence in acidic soil with a pH of <5 (Kisnierien and Lapeikaite, 2015), which inhibits root growth in plants (Frankowski, 2016). The toxicity of Al<sup>3+</sup> under acid soil conditions is mainly due to its high mobility in soil solution, which has toxic effects on various living organisms, contrary to its immobile state as insoluble hydroxide [Al(OH)<sub>4</sub><sup>-</sup>] under neutral soil conditions (Kisnierien and Lapeikaite, 2015). Also, Al toxicity inhibits the plant uptake of nutrients like P (Chen and Liao, 2016). Even though the Al toxicity in acidic soils could affect plant P uptake, the application of 25 mg P kg<sup>-1</sup> as RP alone in Oxisol soils resulted in increased Swiss chard tissue content, indicating lower application rates of RP in Oxisol soil are enough for increased P tissue content. Apart from the treatment with 25 mg P kg<sup>-1</sup> as RP alone, the highest concentration of P in Swiss chard tissue was also observed in the treatments supplied with the highest P level of 50 mg P kg<sup>-1</sup> as pig manure vermicompost.

With the presence of humic acids in the organic amendments used in the present study as recorded in the study of Ajibade et al. (2020) and the goethite present in the Oxisol soil (Manyevere, 2014) used in the present study, the highest P tissue content observed after the application of 50 mg P kg<sup>-1</sup> can be explained by the highest rate of organic amendment used. For example, Yan et al. (2016) prepared goethite–humic acid complexes to investigate their P adsorption characteristics and found that the presence of organic compounds reduces goethite–humic acid complexes' P adsorption capacity. This indicated that a lesser amount of P as pig manure vermicompost would not be enough for the growth of Swiss chard tissue since plants require P during their early stages of growth.

The high K content of Swiss chard tissue after applying cow manure vermicompost at both the application rates of 25 and 50 mg P kg<sup>-1</sup> could be useful in avoiding potassium deficiency of leafy vegetables like Swiss chard. It is possible that the reduced amount of total P observed in Swiss chard tissue after the application of RP-enriched cow manure vermicompost could be associated with the increased K content of Swiss chard tissue that is grown under this treatment. Excess K uptake is reported to result in P uptake blockage by plants (Zone9Garden.com, 2019).

### Effect of RP-Enriched Cow and Pig Manure Vermicompost on the Heavy Metal Content of Swiss Chard Tissue

The most problematic heavy metals in plants are Cr, Cd, Cu, Pb, Ni, and Zn (Jaishankar et al., 2014). The Cu content of Swiss chard tissue ranged between 0.42 and 0.64 mg kg<sup>-1</sup>, which did not exceed the maximum tolerable intake of 5.0 mg kg<sup>-1</sup> allowable by international standards (FAO/WHO, 2014). The highest Cu content of 0.64 mg kg<sup>-1</sup> was observed in the treatment with 50 mg P kg<sup>-1</sup> as cow manure vermicompost, followed by the pig manure vermicompost with a Cu content of 0.63 mg kg<sup>-1</sup>. These results also show that Cu could easily migrate to the Swiss chard tissue in the Oxisol soil after using cow and pig manure vermicompost. As the concentration of Cu in Swiss chard tissue increases, the excess amount of Cu could affect important physiological processes like respiration and photosynthesis (Chetan and Ami, 2015), and therefore, the use of cow and pig manure RP vermicompost at higher concentrations than 50 mg kg<sup>-1</sup> might affect the plant respiration and photosynthesis and, therefore, its growth, and yield.

The Zn content of the Swiss chard tissue was high in most treatments, with a maximum Zn content of 8.50 mg kg<sup>-1</sup>. In all the treatment combinations, including the control treatment, the maximum tolerable intake of 1.0 mg kg<sup>-1</sup> was exceeded (FAO/WHO, 2011), with the highest Zn tissue in the treatment with 50 mg P kg<sup>-1</sup> as cow manure vermicompost. These results explain the high chlorophyll content observed in the Swiss chard tissue in the treatment with 50 mg P kg<sup>-1</sup> as cow manure vermicompost compared with 50 mg P kg<sup>-1</sup> as pig manure vermicompost and RP alone was used. Zn deficiency in plants affects leaf chlorosis by reducing chlorophyll content (Lopez-Mosquera et al., 2000). In all the treatment combinations, the amount of Cr in Swiss chard tissue was below the permissible

level of 2.3 mg kg<sup>-1</sup> (FAO/WHO, 1989). It is worth noting that the Cr increase in Swiss chard planted with RP alone at an application rate of 50 mg P kg<sup>-1</sup> can be explained by the Cr content of 18.04 mg kg<sup>-1</sup> found in the low-grade RP used in the present study, which is higher than other micronutrients found in the RP such as Pb (6.05 mg kg<sup>-1</sup>), Cu (5.85 mg kg<sup>-1</sup>), and Zn (13.22 mg kg<sup>-1</sup>).

Lead concentration did not exceed the safe limit, as Morgan (2001) noted; the normal Pb range should be between 0.20 and 20 mg kg<sup>-1</sup> in edible vegetables. According to the FAO/WHO (1989), the safe limit of Pb in the food source is 0.3 mg kg<sup>-1</sup>. The concentration of Pb was less than this amount in most treatments except in the treatment with 50 mg P kg<sup>-1</sup> as pig manure vermicompost, which was slightly higher (0.34 mg kg<sup>-1</sup>). In the same treatment with 50 mg P kg<sup>-1</sup> as pig manure vermicompost, the lowest chlorophyll content of Swiss chard tissue was recorded, which could be associated with the ability of Pb to reduce plant uptake of chlorophyll essential elements like Fe and Mg, which affects the chloroplast, disturbing the essential enzymatic process for the closing of stomata and photosynthesis (Sharma and Dubey, 2005). This shows that heavy metal concentration through vermicompost differs according to the feed used during vermicomposting. Moreover, the overall low plant uptake of heavy metals in the present study in connection to increased humic substances in the organic P amendments used in the present study (Ajibade et al., 2020) can be explained by the binding ability (e.g., ion exchange, complexation, and physical adsorption) of humic substances for heavy metals and the immobilization of heavy metals through redox reaction (Tang et al., 2014; Yao et al., 2019). These reduced metals show that adding cow and pig manure RP vermicompost to Oxisol soil did not directly affect the Swiss chard plant.

## CONCLUSIONS

Our study concludes that Swiss chard can be grown organically using the best combination of vermicompost made using either cow or pig manure amended with rock phosphate and phosphorus-solubilizing microorganisms. We can conclude that there is limited uptake of heavy metals like Cu, Pb, and Cr with these organic fertilizers except for Zn, which might need to be monitored. Regardless of the growth stage, the pig manure vermicompost mixture used in the study is good for

healthy seedlings and mature Swiss chard plants. Our study can recommend the vermicompost we evaluated as a potential climate-smart technology for growing vegetable crops. Also, with the low cost of raw RP in South Africa and the acidic nature of the soils, vegetables can still be grown after direct application with RP and RP-enriched vermicomposts at lower application rates.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

## AUTHOR CONTRIBUTIONS

SA, HM, and PM conceptualized the study and performed the methodology. SA conducted the formal analysis and contributed in the writing—original draft preparation. SA, HM, AM, and PM conducted the investigation and gathered resources. HM, AM, and PM contributed in the writing—review and editing and supervised the study. All authors contributed to the article and approved the submitted version.

## FUNDING

This research was funded by the National Research Foundation (NRF) Freestanding, Innovation and Scarce Skills (grant no. SFH150708124213) and FoodBev SETA; the Govan Mbeki Research and Development Center (GMRDC) at the University of Fort Hare funded part of the SA bursary through a postgraduate fee waiver.

## ACKNOWLEDGMENTS

We are thankful to the reviewers for their great input in improving our manuscript.

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fsufs.2022.757792/full#supplementary-material>

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