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Nitrogen fertiliser value of products from dehydration of pine bark–human urine mixtures under field conditions

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There is limited information on the agronomic performance of fertiliser products from dehydration of mixtures of urine and solid waste materials under field conditions. This study investigated the effects of products derived from dehydration (glasshouse) of pine bark (PB), biochar, and ash mixed with artificial human urine as sources of nitrogen (N) for spinach (*Spinacia oleracea)* in field conditions. A field trial was conducted at the Ukulinga Research Farm to evaluate the effect of these pine bark urine-based products on spinach growth over two seasons. The products were applied at the recommended N rate for spinach in the first season. The pine bark feedstock, biochar, and ash that were not pretreated with urine were included as reference materials, whereas plots without N and with urea were included as negative and positive controls, respectively. In the second season, the same plots were used without any additional treatment to assess residual effects. In each season, the field trial lasted for approximately 3 months, from 04/06/2024–04/09/2024 and 12/09/2024–04/12/2024. Spinach dry matter yield, nutrient uptake, and tissue micronutrients were determined, together with soil pH, extractable phosphorus (P), and mineral N. The urine-based products improved dry matter yield and N uptake of spinach as compared with the negative control. However, high sodium (Na) concentrations were detected in plant tissues (first season). Residual applications led to an increase in dry matter yield of biochar-U (238 g $plot^{-1}$) compared with the positive control (155.7 g $plot^{-1}$). Residual application of urine-based fertilisers reduced the risk of sodium accumulation, as the initial crop absorbed most of the sodium. These findings suggest that urine-based (biochar-U) fertilisers are a viable and sustainable alternative to mineral N fertilisers. This supports the philosophy of circular economy through resource efficiency, as promising option for smallholder farmers, although Na concentrations need to be monitored.

KEYWORDS

biochar-based fertilisers, nitrogen recovery, residual fertiliser effects, spinach (*Spinacia oleracea*), sustainable nutrient management, urine-derived nutrients

1 Introduction

The application of human urine has been shown to enhance cereal production in field trials (1). Similarly, Morgan (2) reported higher vegetable yields in urine-treated plots compared with unfertilised ones in Zimbabwe. Field trials on wheat also demonstrated increased yields with urine application compared with unfertilised plots (3). Field comparisons with conventional fertiliser show that human urine alone can significantly increase vegetable yields compared with unfertilised controls (snap beans and turnips) (4). Field trials in Niger using human urine increased pearl millet panicle yield by ~170–313 kg ha^{-1} over control across years of farmer managed fields (5). These findings highlight the effectiveness of urine as a fertiliser under field conditions. However, improper handling of urine can lead to pollution due to its high pH, which promotes nitrogen (N) loss through ammonification (6). Stored urine undergoes urea hydrolysis, increasing pH and shifting N urea to ammonia, which can increase volatilisation losses if not managed properly, highlighting the need for controlled handling in fertiliser applications (7). Additionally, the high transportation costs, caused by the large water content (97%) in human urine, make it challenging to use urine effectively for fertilization. As a result, recovering N using various substrates has become an emerging research focus. Dehydrating urine with ash and other products has shown promise in retaining N (8–10).

Sustainable agriculture that aims at reducing the reliance on mineral fertilisers to minimise their adverse environmental impacts is an emerging trend. Nitrogen is a vital nutrient for plant growth and development, playing a key role in chlorophyll synthesis, protein formation, and overall crop productivity (11). However, the efficiency of N fertilisers in agricultural systems is often limited by significant nutrient losses and associated environmental pollution (12). Furthermore, the high cost of mineral fertilisers poses an additional challenge, particularly for smallholder farmers (13). Therefore, there is a pressing need to explore sustainable strategies which are affordable to enhance the value of N fertilisers and optimise crop productivity.

Urine-based products have gained attention as innovative amendments to improve soil fertility and nutrient cycling (14, 15). When properly treated, these products offer a readily available source of N, improving nutrient availability to crops while transforming human and organic waste into sustainable agricultural inputs (16). This research aligns with global efforts to promote circular economy principles, reduce waste, and transition toward more sustainable agricultural practices (16, 17). Preliminary experiments demonstrated that pine bark-based materials (raw, biochar, and ash) effectively recovered N from artificial human urine, rapidly released it when applied to the soil, and enhanced dry matter yield of spinach in a glasshouse study (18). As a results, recovering N using various substrates has become an emerging research focus. Several approaches have been developed to stabilise and recover N from human urine, including mineral-based carriers such as zeolite and struvite precipitation, as well as carbonaceous materials such as urine–biochar. Zeolite retains ammonium primarily through cation exchange, whereas struvite recovers N and phosphorus. Biochar retains N through a combination of adsorption, alkalinity, and physical entrapment during drying and has been shown to enhance N use efficiency following soil application.

Pine bark is characterised by an acidic pH and a relatively stable carbon structure, with carbon stability, porosity, and surface charge increasing as pyrolysis temperature rises (19). In contrast, pine bark ash has a high calcium carbonate equivalent and therefore has potential to ameliorate soil acidity through liming effects (18). Overall, pine bark-derived substrates are low-cost, locally available forestry by-products that support nutrient recovery while simultaneously valorising organic waste streams (20). The findings from our preliminary results demonstrated that these products produced spinach yields comparable with those achieved with mineral N fertiliser when applied at the recommended N rate under a controlled environment. However, the agronomic performance of these products has not yet been tested, as N sources, under field conditions, where environmental factors are uncontrolled, temperatures fluctuate, and plants experience cycles of wetting and drying.

This study evaluated the N fertiliser potential of dehydration products made from mixtures of urine with raw pine bark (PB), biochar, and ash on spinach yield under field conditions. Additionally, the study aimed to assess the residual effects of these urine-based dehydration products on spinach yield, nutrient composition, and soil available N and P under the same field conditions. Spinach is an important food crop that thrives in cool, temperate climates (21–23). In warmer southern regions, spinach is grown during winter, whereas in cooler northern regions, it is sown in spring (24), which makes it an ideal crop for testing the fertiliser value of the urine-based fertilisers.

2 Materials and methods

2.1 Experimental site

The field experiment was conducted at the Ukulinga Research Farm of the University of KwaZulu-Natal (29° 39′ 33.9″ S; 30° 24′ 14″E). This area has warm to hot summers with a mean monthly maximum temperature of 28°C, and mild winters with a mean monthly minimum temperature of 8.7°C, and with annual rainfall of 717 mm. The soil was loam in texture and classified as Westleigh (25), which was translated to Plinthic Acrisols (26). Bulk soil samples were collected from the 0–20-cm depth, mixed, homogenised, air-dried, and sieved (<2 mm), before analysis. The clay content of the samples was estimated using the near infrared reflectance (HTS-XT, Bruker, Germany), whereas total carbon (C) and nitrogen (N) were measured using the LECO Trumac CNS Autoanalyser (LECO Corporation, 3000, Lakeview, Ave, ST, Joseph, MI, USA)). Phosphorus was extracted with the AMBIC-2 method (27) and analysed with a UV/VIS spectrophotometer following the molybdenum blue method (28). Soil pH, exchangeable bases, and

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TABLE 1 Characteristics of the soil used in the study.

Property	Acrisols
Clay (%)	29
Bulk density (g cm^{-3})	1.24
pH $_{(KCl)}$	5.08
Total carbon (%)	1.95
Total nitrogen (%)	0.165
C/N	11.8
Extractable phosphorus (mg/kg)	3.86
Exchangeable potassium (cmolc/kg)	0.397
Exchangeable calcium (cmolc/kg)	8.79
Exchangeable magnesium (cmolc/kg)	7.48
Exchangeable acidity (cmolc/kg)	0.05
Acid saturation (%)	0

acidity were analysed using standard methods (29). The characteristics of the soils are shown in Table 1.

2.2 Urine-based product production and characterisation

Pine bark waste (PB), biochars, and ashes from these wastes were used in this study. The pine bark were ground to<2-mm particle size using a Retsch KG 5657 HAAN (West Germany) machine and stored in plastic bags. Portions of the samples were heated at 750°C for 6 h in a muffle furnace to produce ash. The characteristics of the media are similar to those by Vilakazi et al. (19). Additionally, biochar was produced using a two-container kiln at Ukulinga Research Farm, which was conducted in the Department of Engineering at the University of KwaZulu-Natal, with further details on the link provided in the Supplementary Material. Proximate analysis results for this biochar showed 58.3% volatile matter, 40.8% fixed carbon, and 0.82% ash, and the total C, N, P, and K were 65, 0.32, 0.005, and 0.355%, respectively. The pH was determined in KCl at a ratio of 1:10 (30), and the calcium carbonate equivalent was evaluated following the method by Singh et al. (31). The pH and calcium carbonate (CCE) were 4.32% and 8.92%, respectively. Artificial human urine (AHU) used in this study had 12.2 g L^{-1} urea, 0.17 g L^{-1} uric acid, 0.45 g L^{-1} creatinine, 1.49 g L^{-1} tri-sodium citrate, 3.17 g L^{-1} sodium chloride, 2.25 g L^{-1} potassium chloride, 0.805 g L^{-1} ammonium chloride, 0.17 g L^{-1} sodium bicarbonate, 0.445 g L^{-1} calcium chloride, 0.5 g L^{-1} magnesium sulphate, 1.29 g L^{-1} disodium sulphate, 0.5 g L^{-1} monosodium phosphate, and 0.55 g L^{-1} disodium phosphate (32). The artificial human urine (AHU) contained multiple components, with their concentrations falling within the physiological ranges observed in normal human urine. The use of artificial human urine in this study was aimed at ensuring experimental consistency and control of nutrient composition across treatments. Although artificial urine does not fully replicate the microbial and organic complexity of real human urine (33), it remains a valuable research tool under controlled experimental conditions. Its use allows standardisation of nutrient concentrations and minimises variability associated with donor diet and storage. This approach ensured that observed differences in nutrient recovery and plant response could be attributed to treatment effects rather than inconsistencies in urine composition.

To produce these materials on a large scale, the urine-pine bark, biochar, and ash were dehydrated under glasshouse conditions (G), with the cooling system switched off. A sample of 7 kg of each medium was added initially to plastic basins, followed by AHU application at a rate of 5.1 L m^{-2}, and after each application, the mixtures were stirred and allowed to dehydrate. Total carbon (C) and N were measured. The results for the end products are shown in Table 2.

2.3 Experimental design

The first planting season occurred during the winter season, characterised by lower temperatures and limited rainfall. In contrast, the second planting season took place during the spring to early summer period, which experienced higher temperatures and increased rainfall. During the study period, the rainfall (TE525, Texas Instruments, USA) and air temperature (HMP60, Vaisala, Sweden) were measured and recorded on a datalogger (CR3000, Campbell Scientific, Utah, Logan, USA) every 60 s and total rainfall and average air temperature were calculated daily. The rainfall and temperature data for both seasons are shown in Figure 1.

2.3.1 Field trial experiment (seasons 1 and 2)

The experiment was set up as a randomised complete block design with treatments replicated four times. Treatments included urine-based products derived from raw pine bark, biochar, and ash (PB-U, biochar-U, and ash-U). Negative (No N) and positive (mineral N) controls were also included. The products were applied at the recommended N rate for spinach, equivalent to 100 kg ha^{-1}. The mineral N in the positive control was applied as urea at 100 kg N ha^{-1} for optimum yield. Equivalent amounts of 124 kg P

TABLE 2 Percentage of N recovered from urine and total carbon and N content after dehydration in the glasshouse.

Urine products	pH	Rec. N in urine added	N (%)	C (%)	C: N
PB	6.40^{a}	72.9^{b}	2.02^{a}	36^{b}	18.8^{b}
Biochar	6.88^{b}	81.5^{c}	2.29^{a}	37.2^{b}	16.3^{ab}
Ash	7.05^{c}	65.5^{a}	1.55^{a}	7.13a	4.98^{a}

Values on the same column with different letters indicate significant differences ($p<0.05$).

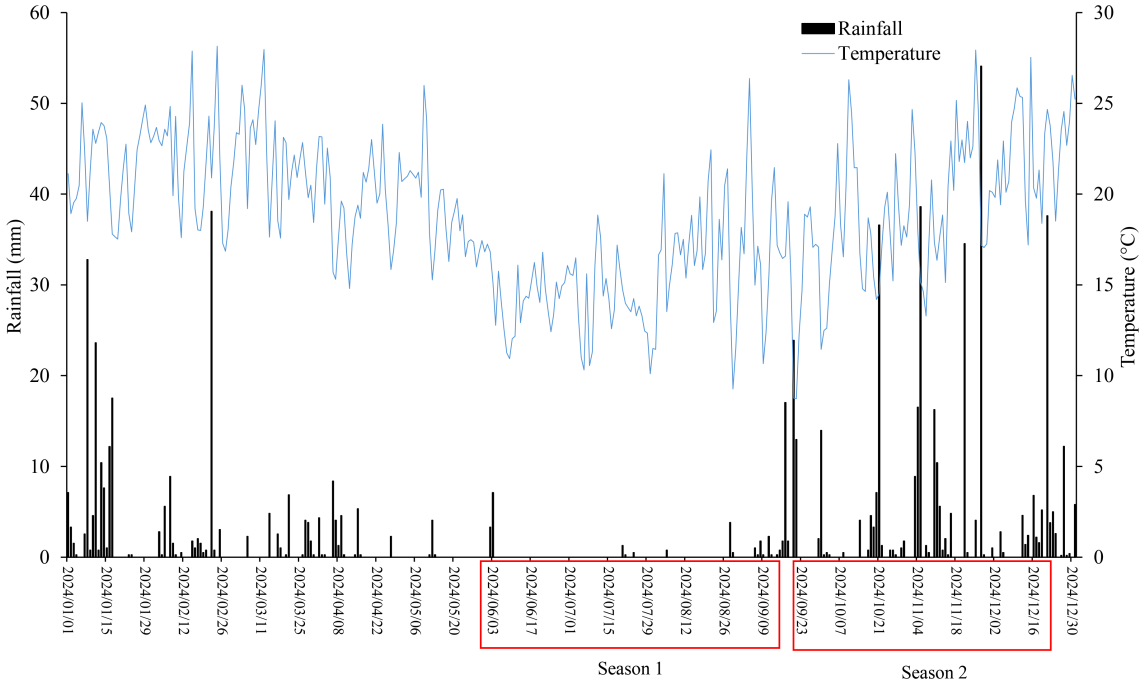

FIGURE 1
Rainfall and temperature for Ukulinga research farm.

ha^{-1} and 117 kg K ha^{-1} were applied to all plots (all treatments) as monocalcium phosphate and KCl, respectively (27). The same materials (pine bark, biochar, and ash), without urine treatment were included as reference materials on adjacent plots in the first season.

The field layout of treatments is shown in Figure 2. In total, there were 32 plots, of which 20 plots had urine-based products and 12 were for the treatments that were not enriched with urine. The 20 plots were 2m × 5m, with 52 plants per plot. The other plots, where un-enriched materials were added, were 2m × 2.5m in size, with 24 plants per plot. The spinach seedlings were planted on the 12th of June 2024 with harvesting done after 6 weeks and harvested on 4th of September 2024 at the end of the trial. Supplemental irrigation was added to make sure water was not a limiting factor. After 6 weeks (42 days), the spinach was harvested by cutting at 1 cm above the soil surface and leaving younger leaves to grow, until after 12 weeks where all the aboveground spinach was harvested within each experimental plot. The biomass was then dried at 60 °C for 3 days to determine dry weight. The plant tissue samples harvested at 6 and 12 weeks were combined to form a composite sample and then ground (<1mm) using a blender before analysis of total N, P, Ca, Mg, Na, K, Zn, Mn, Cu, and Fe. The N and P uptakes were also calculated as the product of concentrations and dry matter yield. At the end of the experiment (12 weeks), soil samples were collected

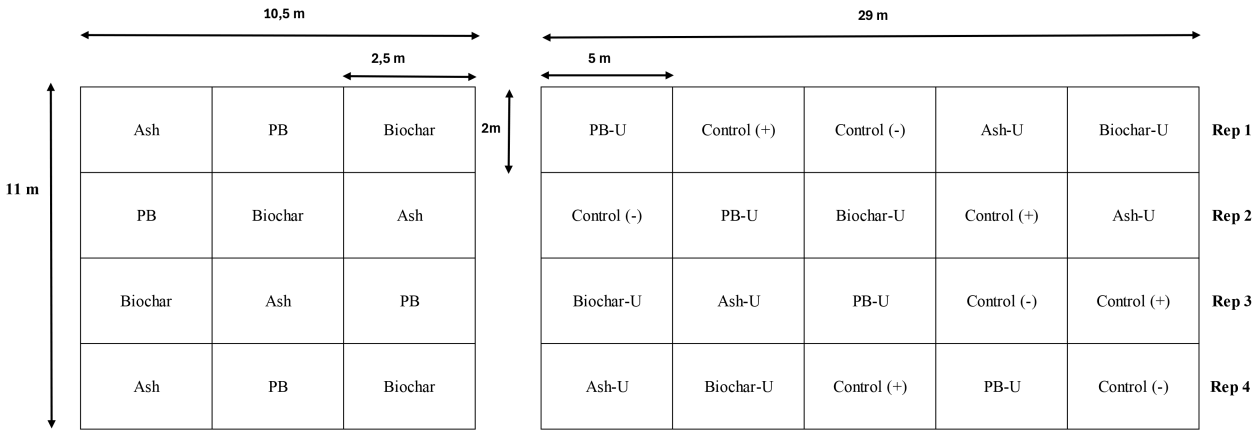

FIGURE 2
Experimental layout for the first planting season.

from the 0-20-cm depth and analysed for mineral-N, pH, and extractable P. The soils (10 g) were oven dried overnight at 105°C to correct mineral-N and available P results for moisture. In the second season, the seedlings were planted in the same plots (residual effects), except the reference plots, and managed the same way except that there was no additional application of the treatments. The spinach seedlings were planted on the 12th of September 2024 and harvested on the 4th of December 2024.

2.4 Analyses

Total N was analysed, on a 0.2-g ground sample (<250 µm), by dry combustion using the LECO Trumac (CNS) autoanalyser (LECO Corporation, 3000, Lakeview, Ave, ST, Joseph, MI, USA). For other analyses, the ground spinach samples (0.5 g) were placed into crucibles, treated with 10 ml of concentrated HNO_3 and HCl (1:3) (34), and digested for 1 h using a MARS CEMS microwave digester, before analysis of phosphorous (P), calcium (Ca), magnesium (Mg), potassium (K), Iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu) concentrations using an inductively coupled plasma atomic emission spectrometer (Varian 720-ES ICP-AES). The N and P uptakes were calculated using Equation 1.

$$\text{N/P uptake (g plot}^{-1}) = \text{dry matter yield (g plot}^{-1}) * \frac{(N \ / \ P \ \ concentration)}{100} \quad (1)$$

2.4.1 Soil pH, mineral-N, and available P analysis

Soil pH, mineral-N (NH_4- and NO_3-N), and extractable P were analysed in the residual soils after each harvest. Soil pH was measured in KCl at a ratio of 1:5 using 1 M KCl. Ammonium- and nitrate-N were extracted using 2 M KCl and measured using a Gallery auto analyser (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Extractable P was analysed using the molybdenum blue method (28), after extraction with AMBIC-2 (27).

2.5 Statistical analysis

All data of spinach yield, macronutrient uptake and micronutrient concentration, and soil pH, mineral N, and available P were subjected to the one-way analysis of variance (ANOVA) using GenStat 18th edition. Separation of treatment means was done using least significant difference (LSD) at $p < 0.05$. The Tukey HSD test was also used to separate treatment means at $p < 0.05$.

3 Results

3.1 Dry matter yield and nitrogen uptake

Application of urine-based products significantly increased spinach yield in the first season, with higher dry matter yield and N uptake compared with the negative control (Figures 3A, 4A). The positive control produced the highest dry matter yield (767 g plot^{-1}) and N uptake (25.6 g plot^{-1}), values that were comparable with biochar-U but significantly greater than those recorded for pine bark-U and ash-U. No significant differences were observed among the urine-enriched treatments. However, pine bark products enriched with urine resulted in significantly higher dry matter yield and N uptake than their corresponding non-enriched products, which did not differ from the negative control.

Under residual conditions, only the biochar-U treatment maintained higher dry matter yield and N uptake relative to both controls (Figures 3B, 4B). Although N uptake did not differ significantly among urine-enriched treatments, dry matter yield in the biochar-U treatment was significantly higher than that observed for pine bark-U.

3.2 Uptake of P and K and tissue concentrations of Ca, Mg, and Na

The uptake and mineral concentration in spinach showed varying results across different treatments (Table 3). The biochar-U, pine bark-U, and the positive control showed significantly higher P uptake compared with products without urine enrichment and negative control. All products with urine enrichment were not different, and only the biochar-U had higher P uptake than the negative control (Table 3). All products with urine enrichment showed significantly high K uptake compared with negative control. There were no treatment responses of Ca concentration when compared with the negative control (Table 3). However, the products without urine enrichment had lower K uptake than those with urine enrichment and the positive control. The Mg concentration is higher in the positive control, the urine-enriched products, and the negative control (Table 3). The urine-derived products had significantly higher Na concentration than all other treatments, which were not significantly different.

3.3 Uptake of P and K and tissue concentrations of Ca, Mg, and Na for second season residual

In the experiment to determine residual effects, K uptake was higher in the biochar-U treatment than the negative control, whereas the tissue Mg concentration in the same treatment (biochar-U) was higher than in the positive control (Table 4). There were no significant differences in the treatments for P uptake, tissue Ca, and Na (Table 4).

3.4 Cu, Zn, Fe, and Mn concentration for the first season

There were no significant differences in copper (Cu), zinc (Zn), manganese (Mn), and iron (Fe) concentrations in spinach plant tissues across treatments (Table 5).

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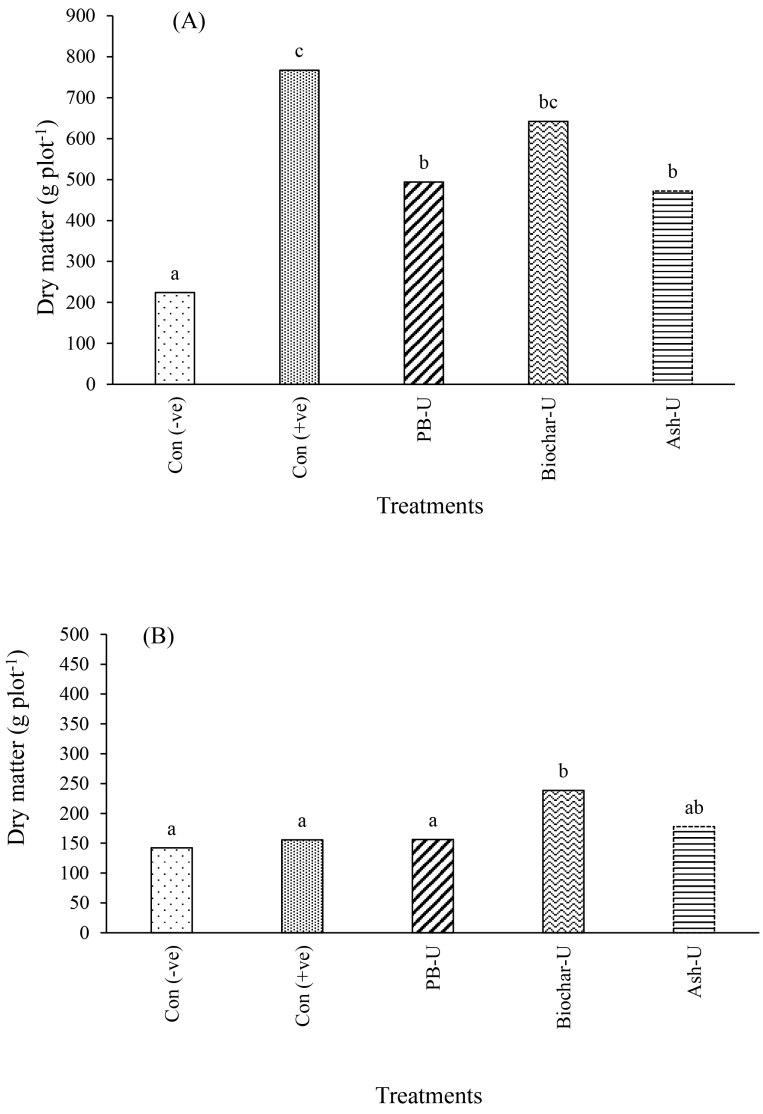

FIGURE 3
Dry matter yield of spinach as affected by different PB-urine-based products and seasons **(A)** experiment 1, **(B)** experiment 2. PB-U, PB biochar-U, and PB ash-U products of pine bark, pine bark biochar from the kiln, and pine bark ash, respectively, dehydrated in the glasshouse. PB, PB biochar, and PB ash-U products of pine bark, pine bark biochar from the kiln, and pine bark ash, respectively, with no urine enrichment. There were no statistically significant differences between the reference materials and the negative control.

3.5 Cu, Zn, Fe, and Mn concentrations for the second season

In the residual experiment, the tissue concentrations of Cu, Zn, Fe, and Mn in spinach plant tissues followed a similar trend, where there were no significant differences within the treatments (Table 6).

3.6 Soil pH and extractable P after spinach harvest

In the first season, the soil pH in the ash-U treatment was higher than the pine bark-U treatment and both the controls, whereas there were no significant differences with biochar-U (Table 7). Similarly, extractable P was significantly similar for all treatment with urine, with ash-U and biochar-U being higher than all other treatments.

3.7 Residual soil characteristics for the second season

In the experiment to determine the residual effects, the soil pH in the ash-U treatment was higher than the pine bark-U treatment, whereas there were no significant treatment differences on extractable P, ammonium-N, nitrate-N, and mineral-N concentrations (Table 8).

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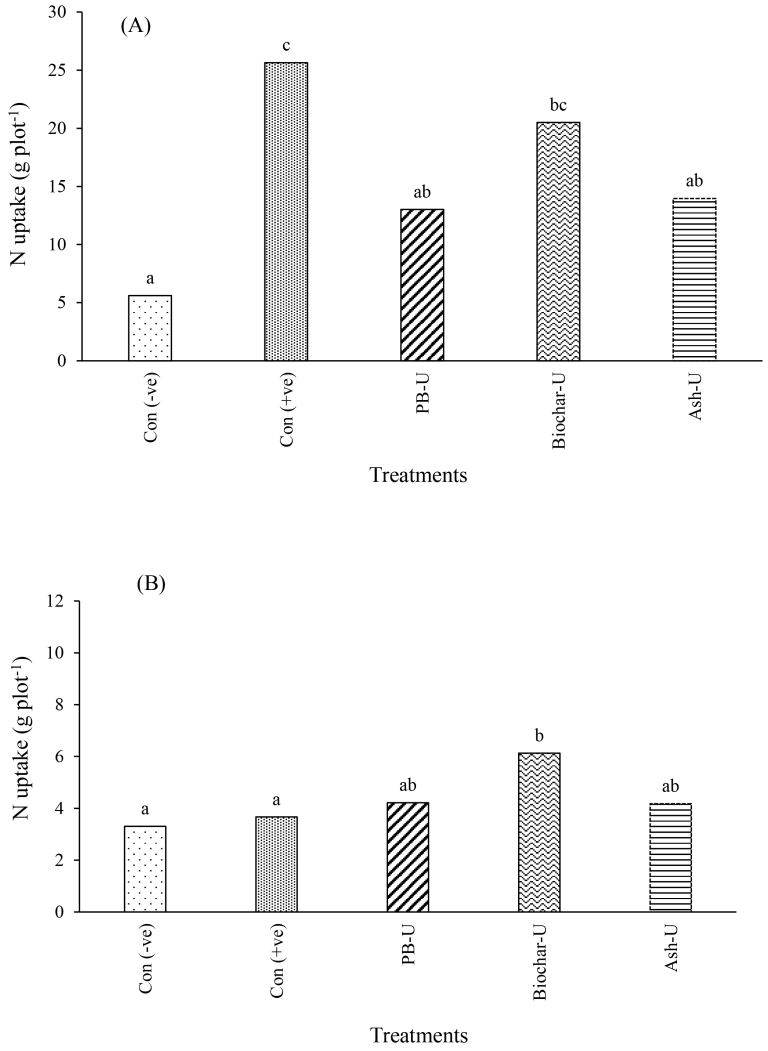

FIGURE 4
The nitrogen uptake of spinach as affected by different PB-urine-based products and seasons **(A)** experiment 1 and **(B)** experiment 2. PB-U, PB biochar-U, and PB ash-U products of pine bark, pine bark biochar from the kiln, and pine bark ash, respectively, dehydrated in the glasshouse. PB, PB biochar, and PB ash-U products of pine bark, pine bark biochar from the kiln, and pine bark ash, respectively, with no urine enrichment.

TABLE 3 The uptake of P and K and Ca, Mg, and Na concentrations of spinach when PB-non-enriched and enriched with urine were applied during the first planting season.

Treatments	Tissue uptake (g plot^{-1})		Tissue concentration (%)		
	P	K	Ca	Mg	Na
Con (−ve)	1.51^{a}	8.74^{a}	1.16^{a}	1.45^{a}	1.84^{a}
Con (+ve)	3.51^{ab}	20.9^{b}	1.06^{a}	1.88^{b}	1.61^{a}
PB-U	3.50^{ab}	21.0^{b}	0.941^{a}	1.09^{a}	2.47^{bc}
Biochar-U	3.95^{b}	23.2^{b}	1.05^{a}	1.35^{a}	2.92^{c}
Ash-U	2.53^{ab}	18.5^{b}	0.949^{a}	1.20^{a}	2.45^{bc}

No significant differences were observed between the reference materials and the control. PB-U, biochar-U and ash-U- products of pine bark, pine bark biochar from the kiln and pine bark ash, respectively, dehydrated in the glasshouse.
Values on the same column with different letters indicate significant differences ($p<0.05$).

TABLE 4 The residual uptake of P and K and Ca, Mg, and Na concentrations of spinach planted on plots previously treated with pine bark materials enriched with urine.

Treatments	Tissue uptake (g plot^{-1})		Tissue concentration (%)		
	P	K	Ca	Mg	Na
Con (−ve)	0.829^{a}	3.27^{a}	0.770^{a}	0.70^{ab}	1.73^{a}
Con (+ve)	0.867^{a}	3.79^{ab}	0.711^{a}	0.595^{a}	1.60^{a}
PB-U	0.722^{a}	3.68^{ab}	0.792^{a}	0.735^{ab}	1.88^{a}
Biochar-U	1.18^{a}	5.92^{b}	0.805^{a}	0.785^{b}	1.51^{a}
Ash-U	1.01^{a}	5.43^{ab}	0.743^{a}	0.691^{ab}	1.86^{a}

PB-U, biochar-U and ash-U- products of pine bark, pine bark biochar from the kiln and pine bark ash, respectively, dehydrated in the glasshouse.
Values on the same column with different letters indicate significant differences ($p<0.05$).

TABLE 5 The Cu, Zn, Fe, and Mn concentrations of spinach when non-enriched and enriched with urine were applied during the first planting season.

Treatments	Tissue concentration mg kg^{-1})			
	Cu	Zn	Fe	Mn
Con (–ve)	15.9^{a}	247^{a}	101^{a}	1,371^{a}
Con (+ve)	15.1^{a}	199^{a}	126^{a}	1,292^{a}
PB-U	19.2^{a}	176^{a}	92.6^{a}	1,110^{a}
Biochar-U	11.3^{a}	203^{a}	159^{a}	926^{a}
Ash-U	11.1^{a}	191^{a}	158^{a}	1,171^{a}

The optimum concentrations: Cu= 5–26 ppm; Zn= 25–301 ppm; Fe= 60–201 ppm and Mn= 30–251 ppm (35). No significant differences were observed between the reference materials and the control. PB-U,biochar-U, and ash-U products of pine bark, pine bark biochar from the kiln, and pine bark ash, respectively, dehydrated in the glasshouse.
Values on the same column with different letters indicate significant differences ($p<0.05$).

TABLE 6 The residual Cu, Zn, Fe, and Mn concentrations in tissue of spinach planted on plots previously treated with pine bark materials enriched with urine.

Treatments	Tissue concentration mg kg^{-1})			
	Cu	Zn	Fe	Mn
Con (–ve)	15.0^{a}	105^{a}	383^{a}	639^{a}
Con (+ve)	15.5^{a}	105^{a}	671^{a}	698^{a}
PB-U	16.3^{a}	101^{a}	563^{a}	812^{a}
Biochar-U	16.0^{a}	124^{a}	670^{a}	744^{a}
Ash-U	13.4^{a}	107^{a}	451^{a}	720^{a}

PB-U, biochar-U, and ash-U products of pine bark,pine bark biochar from the kiln, and pine bark ash, respectively, dehydrated in the glasshouse.
Values on the same column with different letters indicate significant differences ($p<0.05$).

4 Discussion

The higher dry matter content in treatments with urine-based products, compared with the negative control and non-enriched products during the first season, was associated with greater nitrogen (N) availability and higher uptake of N, P, and potassium (K). This association is reflected in the similar trends observed for dry matter yield, N uptake (Figure 3A), and P and K uptake (Table 3). While these results indicate improved plant yield in urine enriched treatments, they show correlations rather than direct causation.

The N supplied by urine during the enrichment and dehydration could have been in the form of urea. Upon soil application, urea hydrolysis and subsequent nitrification are known to increase mineral N availability under moist conditions (36). However, these processes were not quantified in the present study and are therefore discussed as mechanisms supported by existing literature. The enhanced N uptake observed under urine-enriched treatments is consistent with this interpretation. Similar responses have been reported by Schmidt et al. (37), who reported a fourfold increase in pumpkin yield when using urine-impregnated *eupatorium adenophorum* biochar on a silt loam compared with the control, supporting the potential of urine-based amendments to improve crop growth.

TABLE 7 Soil pH and extractable P concentration after spinach harvest as affected by application of non-enriched and enriched with urine during the first planting season.

Treatments	pH $_{(KCl)}$	Extract. P (mg kg^{-1})
Con (–ve)	4.75^{a}	137^{a}
Con (+ve)	4.57^{a}	129^{a}
PB-U	4.68^{a}	153^{ab}
Biochar-U	4.95^{ab}	228^{b}
Ash-U	5.30^{b}	226^{b}

No significant differences were observed between the reference materials and the control. PB-U, biochar-U, and ash-U products of pine bark, pine bark biochar from the kiln, and pine bark ash, respectively, dehydrated in the glasshouse.
Values on the same column with different letters indicate significant differences ($p<0.05$).

The similarity in dry matter yield and N uptake in biochar-U and the positive control during the first season suggested that, under the specific conditions of this field trial and at the applied (recommended N rate), biochar-U performed comparably with mineral fertiliser. While this highlights the agronomic potential of urine-enriched biochar, this finding should be interpreted with caution. The experiment was limited to a single site, crop, and application rate and did not assess N losses, or performance under varying environmental conditions. Consequently, biochar-U should not be considered a direct replacement for urea, but rather a promising alternative N source requiring further analysis across different crops and environmental conditions.

In contrast, the lower dry matter yield in products without urine enrichment, despite receiving the same added N rate, may reflect limited short-term N availability and the form of N in the products, which may have been unavailable. This may be related to the relatively high C:N ratio of raw pine bark materials, which can promote microbial immobilisation and delay N mineralisation. However, mineral N, decomposition rates, and immobilisation processes were not measured in this study. Considering that P and K were applied at the same recommended rates across all treatments, the higher P and K uptake observed in urine-enriched treatments and the positive control is likely linked to increased biomass production associated with higher N availability. This suggests that improved N uptake enhanced overall nutrient uptake rather than indicating a direct fertiliser effect of urine-based products on P and K supply.

During the second season (residual effect), biochar-U maintained higher dry matter yield compared with other treatments. The residual response coincides with increased N uptake (Figure 4B) and K uptake and Mg concentration (Table 4), suggesting continued nutrient availability. Biochar-based fertilisers are known to be slower nutrient release and longer residence times in soil (38, 39), which may partly explain this response. However, alternative mechanisms such as moisture retention or physical soil structure effects associated with biochar cannot be excluded, as these were not measured.

The high Na concentration in spinach tissue fertilised with urine-enriched treatments during the first season likely reflected the high Na content of the urine-based amendments. Although Na was

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TABLE 8 Residual soil pH and extractable P concentration after harvest of spinach planted of plots previously treated with materials enriched with urine.

Treatments	pH $_{(KCl)}$	Extract. P	Ammon. -N	Nitrate-N	Mineral-N
Con (−ve)	4.64^{ab}	158^{a}	0.136^{a}	4.09^{a}	4.43^{a}
Con (+ve)	4.65^{ab}	198^{a}	0.158^{a}	5.51^{a}	5.87^{a}
PB-U	4.61^{a}	180^{a}	0.199^{a}	4.18^{a}	4.38^{a}
Biochar-U	4.69^{ab}	124^{a}	0.129^{a}	3.87^{a}	4.00^{a}
Ash-U	4.97^{b}	117^{a}	0.155^{a}	3.46^{a}	3.62^{a}

PB-U, biochar-U, and ash-U- products of pine bark, pine bark biochar from the kiln, and pine bark ash, respectively, dehydrated in the glasshouse.
Values on the same column with different letters indicate significant differences ($p<0.05$).

not measured directly on urine-enriched products in this study, literature reports and the composition of the artificial human urine used indicate that it contained high Na levels. Although human urine naturally contains high levels of Na (0.9%) (7, 40), the artificial urine used in this study contained lower Na concentrations (0.22%). The absence of yield reduction despite high tissue Na concentration is consistent with the moderate salt tolerance of spinach (41, 42). However, the lack of yield effects and lower concentration during the second season should not be interpreted as evidence that repeated application poses no sodicity risk. Soil exchangeable sodium percentage (ESP) and long-term Na accumulation were not assessed, and the short duration of the study limits conclusions to long term. Monitoring of soil and plant Na concentrations is therefore recommended where urine-based fertilisers are applied repeatedly. Sodium concentrations in leafy vegetables above 0.5% of dry matter can begin to affect crop quality or taste, although toxicity to humans typically occurs at much higher intake levels than those provided by a single serving of spinach (43). Based on the Na concentrations measured in spinach tissue in this study, occasional consumption of the crop is unlikely to pose a health risk when included as part of a balanced diet. However, given that the elevated tissue Na likely reflects the high Na content of the urine-based fertilisers, repeated consumption of such crops could contribute to overall dietary Na intake. This is particularly relevant for individuals on sodium-restricted diets, where cumulative exposure should be considered.

The Ca concentration in plant tissue did not differ within the treatments, which signifies that little was being contributed by the urine-based products. The copper (Cu) content in spinach remained within the permissible limit (35), whereas zinc (Zn) and iron (Fe) exceeded recommended thresholds in the first and second seasons, respectively, and manganese concentrations (Tables 3 and 4) exceeded the tissue threshold of 500 ppm (35, 44). Elevated Mn concentrations may be associated with soil acidity and enhanced Mn solubility, potentially influenced by acidic functional groups in pine bark materials or nitrification processes following urine application (45, 46). Additionally, the influence of inherent soil properties associated with plinthic acrisols.

Excess soil Mn, Zn, and Fe can lead to bioaccumulation in plants (47) and negatively impact plant functions by inhibiting chlorophyll biosynthesis, reducing photosynthesis rates (48), and impairing CO_2 assimilation and stomatal conductance (49). High Mn levels can also disrupt the uptake, redistribution, and utilization of essential nutrients such as N, P, Ca, Mg, and Fe, all of which are critical for normal plant growth (50). Consequently, Mn toxicity can lead to reduced biomass yield. However, spinach is known as a hyperaccumulator of heavy metals and toxic ions (51), which may explain its ability to maintain high biomass under elevated micronutrient concentrations. Similar observations have been reported under wastewater irrigation, where increased Mn availability did not reduce spinach yield (51, 52). Although high, the Mn concentrations remained below phytotoxic levels (53). The concentrations of micronutrients in spinach tissue following application of urine-based products were within permissible limits for human consumption, as defined by WHO/FAO guidelines (54).

The higher soil pH observed under ash-U during both seasons highlighted the liming potential of ash-derived products. Increased soil pH during the first season coincided with higher extractable P, suggesting enhanced P availability (31, 55). However, whereas higher extractable P was observed in ash-U and biochar-U treatments, this did not consistently translate into higher plant P uptake or yield across seasons, indicating that soil chemical changes alone do not guarantee proportional crop responses. The high extractable P remaining in the soil with the biochar-U treatment, after harvesting in the first season, may have contributed to increasing P uptake and dry matter yield observed in the residual experiment during the second season.

5 Conclusion

The application of pine bark urine-based products increased nitrogen uptake, dry matter yield in spinach (*Spinacia oleracea)*, soil pH, and available phosphorus (P) relative to the negative control. When biochar enriched with urine is applied, it has a positive effect on spinach yields, making it a promising option for smallholder farmers. Additionally, applying ash-U raises soil pH and increases extractable P availability. The ash-U can be used to improve acidic soils by acting as a liming agent while also enhancing P availability. However, using pine bark urine-based products can lead to an increase in sodium (Na) concentration in spinach tissue. To

minimise harvest losses, farmers are advised to plant salt-tolerant crops during the first growing cycle when using urine-based products for the first time.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

SV: Data curation, Formal analysis, Funding acquisition, Methodology, Software, Writing – original draft, Writing – review & editing. AO: Data curation, Investigation, Supervision, Visualization, Writing – review & editing. PM: Conceptualization, Data curation, Formal analysis, Supervision, Validation, Visualization, Writing – review & editing.

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Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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