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# Utilization of phosphogypsum and red mud in alfalfa cultivation

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ABSTRACT

In this work, the utilization of phosphogypsum (PG), a waste coming from the manufacture of phosphate fertilizers, as fertilizer for alfalfa (*Medicago sativa* L.) crops was investigated using pot experiments. The objective of this study was to evaluate the effects of both phosphogypsum and red mud (RM) in two soils representative of the pasture production area in Southern Spain. The morpho-physiological parameters of biomass, plant height, number of stems and number of leaves, as well as the chemical parameters of soil content, were measured. High doses of PG inhibited seed germination in some treatments. In addition, the treatment substrate (2550 g soil + 50 g $kg^{-1}$ PG + 100 g $kg^{-1}$ RM) also affected seed germination, possibly due to the large amount of RM. The application of PG and RM to the soil increased the availability of important nutrients for alfalfa, such as phosphorus (P), calcium ($Ca^{2+}$) and magnesium ($Mg^{2+}$). The results demonstrate that the treatment with PG significantly improved the uptake of P in alfalfa.

## 1. Introduction

Two types of residues are produced during the processing of phosphoric acid (PA) ($H_3PO_4$) and extraction of alumina, which are phosphogypsum (PG) and red mud (RM), respectively. Approximately 300 million metric tons of PG are produced globally each year [1]. On the other hand, RM is a waste product generated during the extraction of alumina from bauxite ore, and it is generated at a rate of up to 175.5 million tons per year [2]. Both PG and RM have the potential to be repurposed or utilized in various applications, depending on their chemical and physical properties [3].

Most of the PG generated is disposed of by dumping it in large stacks, which are often located in coastal areas near the factories that produce it. This disposal method can have negative environmental impacts, as PG is exposed to weathering agents, such as wind and rainfall, which can cause it to break down and release of harmful substances into the surrounding environment. This can lead to severe environmental damages, including contamination of soil and water bodies, by both heavy metals and natural radionuclides and their subsequent bioaccumulation in marine fauna and animal species [4,5]. PG presents high concentrations of toxic trace elements, posing potential health and environmental risks such as Cd, Cr, Cu, Zn, Pb, As, and Hg [ [6–8]]. Although, all metals at high concentrations can induce toxicity in humans, it's noteworthy that As, Hg, Cd, and Pb have no known biological essential function and exhibit toxicity

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even at relatively minimal doses [9]. Specifically, non-degradable pollutants like Cd and Pb carry inherent risks to human health, manifesting a range of degenerative effects including implications for the central nervous system, gastrointestinal disorders, and potential carcinogenicity [10]. On the other hand, the radioactivity can affect several organs and lead to many diseases, including cancer [11–13]. Radioactive elements such as uranium can be intake via inhalation, orally, or through dermal pathways, and even bioaccumulate in cells [14].Therefore, it is important to consider alternative methods of disposal or utilization for PG in order to minimize these negative impacts [15]. Despite the potential uses of PG, only about 15% of global production is recycled [16].

In recent times, PG have been extensively used in agriculture as a valuable aid in restoring both acidic and alkaline soils, as well as a plant nutrient source, soil amendment for saline conditions, and an enhancer for soil physical properties like permeability and structure [17–19]. The beneficial impact of PG on acidic soil rehabilitation is commonly linked to its ability to decrease the levels of mobile aluminum and sodium [17,20]. All these effects can contribute to increasing plant biomass yield, or the amount of plant material produced by a plant [21]. However, the downside of using PG in soil treatments is an elevation in soil radioactivity, often leading to levels that exceeds internationally regulated thresholds. In addition, high mobility of the heavy metals contained in PG must also be considered, such as cadmium and lead, which have specific regulations within the European Union (EC Regulation No. 466/2001). Thus, it is necessary to control the use of PG in agriculture, since its uncontrolled use could lead to a public health problem due to the potential toxicity of the food produced. PG is currently considered a NORM according to both European Union and IAEA regulations (IAEA, 2003; Directive, 2013/59/Euratom) [22]. Naturally Occurring Radioactive Materials (NORM) are substances that contain naturally occurring radioactive nuclides, or atoms with unstable nuclei that emit radiation as they decay. These nuclides can be found in certain rocks, minerals, and fertilizers, and they include radionuclides of uranium, thorium, radium, radon, lead, and polonium. Therefore, it is important to carefully manage and regulate the handling and disposal of NORM in order to minimize these risks [23,24].

RM is an insoluble product manufactured in the extraction of alumina by digesting bauxite at high temperature and pressure. RM is generated in large quantities and ends up piled up in natural areas in Saudi Arabia, in the same way as PG [25]. Consequently, about 160 million tons of RM are being generated annually worldwide [26]. This practice results in a serious environmental problem, as RM, which has a very basic pH (about 12), alters soils and soil biological activity.

Soils are an essential part of terrestrial ecosystems, as they play a fundamental role in agricultural production and ecological stability [27]. Excess exposure to soil contamination with toxic elements affects plant growth and physiological processes by disrupting nutrient uptake [28], inhibiting plant growth [29], and decreasing seed germination [30]. Phytoremediation is a widely used *in situ* remediation of soils contaminated with potentially toxic elements due to its simple operation, low cost, and environmental safety [31]. Phytoremediation is the most promising phytotechnology for the remediation of metal-contaminated soils [32]. Phytoremediation can be carried out through five different strategies: phytoextraction, phytostabilization, phytovolatilization, phytofiltration and phytotransformation [31]. The two main strategies for metal phytoremediation are phytoextraction and phytostabilization. In the former case, plants can extract metals from the soil, while, in latter case, they reduce metal bioavailabilities in soil and their phytouptake [25]. Therefore, phytoextraction is a technique that uses plants for the removal of potentially toxic elements from contaminated soils by accumulating these elements in the aerial parts [33]. RM is used in various fields, including soil remediation [34]. Several studies have shown that alfalfa (*Medicago sativa* L.) can tolerate heavy metals without their growth being impacted [35,36], with alfalfa being a reference crop, as it shows an excellent accumulation capacity and strong resistance against soil contamination with toxic elements, thus it is a preferential candidate for phytoremediation when grown in soil contaminated with toxic elements [31].

Alfalfa is a type of legume that is known for its fast growth rate and high biomass productivity. It is an important perennial forage crop that grows in various parts of the world [37,38] and it is relatively easy to grow, with an extension of 32 million hectares worldwide, with great economic value, owing to its high biomass yield and quality forage, wide adaptability to different environments, nitrogen fixation capacity and soil improvement value [39]. Alfalfa has a high phosphorus (P) requirement [40] and yield varies depending on soil P conditions [41]. Alfalfa is a crop that can tolerate a moderately low-quality water supply [42]. It is widely cultivated around the world and is relatively easy to grow. In addition to its high productivity, alfalfa plants are also known for their ability to accumulate large amounts of persistent toxic elements, particularly in their root systems. This ability to take up and accumulate toxic elements makes alfalfa plants a potential tool for the remediation of contaminated soil. Due to the fact that PG is an acidic material (pH $\approx$ 2), and RM is very alkaline (pH $\approx$ 12), the aim of this study was to combine the use of both wastes to reuse and valorize them in the cultivation of plant species. The effect of the different combined doses of PG and RM on the biomass generated by alfalfa plants and the physiological development of the plant were studied. The utilization of PG and RM as soil amendments and mineral fertilizers in soils of the province of Cádiz for alfalfa planting were investigated by pot experiments. The transfer of pollutants from these wastes into the alfalfa crop is out of the scope of this paper.

## 2. Materials and methods

### 2.1. Materials and site location

Alfalfa, a perennial and herbaceous forage legume, was used in this experiment. The seeds were supplied by a seed company and had a 1000 grain weight of 2.5 g and a germination rate of 95%. This study was carried out in a greenhouse at the University of Huelva (Spain), in Carmen Campus (37°16′N latitude, 6°55′W longitude and 36 m above sea level), under conditions of natural light and temperature, between October 2020 and May 2021. Alfalfa (*Medicago sativa* L., cultivar 'Victoria') was sown in October 2020 (week one), with 20 seeds in every plastic pot (10 cm × 20 cm x 2.5 cm), filled with 3 kg of substrate.

**Table 1**
Chemical characteristics of soil, phosphogypsum (PG) and red mud (RM) before mixing to obtain the different substrates.

| Element | Soil | PG | RM |
|---|---|---|---|
| Al (%) | 2.05 | 0.10 | 8.87 |
| Ca (%) | 1.64 | 8.65 | 5.44 |
| Fe (%) | 1.52 | 0.03 | 10.5 |
| K (%) | 0.47 | 0.02 | 0.09 |
| Mg (%) | 0.33 | <0.0025 | 0.17 |
| Na (%) | 0.12 | 0.11 | >3 |
| P (%) | 0.43 | 0.28 | 0.097 |
| S (%) | 165.63 | 7.02 | 1.06 |
| Si (%) | 45.78 | 0.16 | – |
| Ba (ppm) | 104.58 | 42.50 | 130 |
| Cr (ppm) | 33.75 | <25 | 394 |
| Mn (ppm) | 403.13 | <25 | 211 |

**Table 2**
Mass proportions (soil/phosphogypsum (PG)/red mud (RM)) of the different substrates in relation to a total of 1000 parts for the resulting treatments.

| Code | Treatments | Soil | PG | RM |
|---|---|---|---|---|
| 1-0-0 | 1 | 1000 | 0 | 0 |
| 1-5-0 | 2 | 995 | 5 | 0 |
| 1-5-10 | 3 | 985 | 5 | 10 |
| 1-5-100 | 4 | 895 | 5 | 100 |
| 1-50-0 | 5 | 950 | 50 | 0 |
| 1-50-10 | 6 | 940 | 50 | 10 |
| 1-50-100 | 7 | 850 | 50 | 100 |
| 1-300-0 | 8 | 700 | 300 | 0 |
| 1-300-10 | 9 | 690 | 300 | 10 |
| 1-300-100 | 10 | 600 | 300 | 100 |

The soil was collected from the area around Cádiz in the south of Spain (36°47′N latitude, 5°30′W longitude and 386 m above sea level), with pH = 7.1, and it was homogenized by quartering [43]. The total soil weight was 250 kg. The soil was amended with PG from the Huelva piles (37°15′N latitude, 6°54′W longitude and 10 m above sea level) located next to the city of Huelva (SW Spain) [22,44]. RM waste was obtained from Saudi Arabia and has been used in some treatments to neutralize the acidity of PG [26,45]. Chemical characteristics of soil, PG and RM are summarized in Table 1. The chemical properties of PG showed high Ca, P and S contents, whereas RM showed high Ca, Fe and Na contents (5.4%, 10.5% and 3.0 %, respectively) (Table 1).

Soils treated with different proportions of waste, i.e., PG and RM, were used, generating the different types of substrates. Thus, the different substrates gave rise to the different types of treatments. Treatment 1 was used as control, and it only contained soil from the Cádiz province, without waste. Treatments 2, 3 and 4 had 0.5% PG, treatments 5, 6 and 7 had 5% PG, and treatments 8, 9 and 10 had 30% PG. Treatments 1, 2, 5 and 8 were not mixed with RM, while treatments 3, 6 and 9 contained 1% RM, and treatments 4, 7 and 10 contained 10% RM (Table 2).

### 2.2. *Plant establishment*

Plastic pots (10 cm × 20 cm x 2.5 cm), each containing 3000 g of substrate, were seeded with 20 seeds of alfalfa per pot. A total of 30 pots (ten treatments, with three replicates each) were sown at the beginning of October 2020 (week one), under optimal temperature conditions as identified for alfalfa (soil temperature >10 °C). The plastic pots filled with the substrates were placed on polyethylene plates (40 cm in diameter; one pot per plate) on the ground, and they were watered with distilled water on demand, when the pot dishes had no water in them. The substrate of each pot was mixed before sowing with 2 g of a commercial NPK-S (15-15-15 + 25 S) fertilizer, together with micronutrients (21.43 mg Zinc and 8.18 mg Boron). All pots were watered on the same day to avoid leaching. Alfalfa seeds germinated between November 2nd and 13th. The mean temperature and luminosity during the experiment were 20.8 °C and 170 μmol/s/m$^2$, respectively. Plant growth was estimated by measuring plant height, number of stems and number of leaves. The height of the plant was determined every two weeks, and the first measurement was taken 15 days after the germination of the alfalfa. For this purpose, ten plants were selected from each pot, and the height was measured using a 30 cm ruler. The plant height value for each pot was the result of the average of the measurements of the 10 plants recorded every two weeks. Measurements were repeated 12 times until the first and only cutting 224 days after sowing.

The drainage was analyzed two times (in winter and spring) to verify the possibility of their discharge into the environment, and pH and electrical conductivity (EC) were recorded. Alfalfa samples were collected from the selected pots at the end of the experimental period (week 32) for the analysis of their multielemental composition.

**Table 3**
ANOVA soil pH and CE recorded during the crop cycle (winter and spring).

| Treatments (T) | pH[a] | EC[a] (mS $cm^{-1}$) | pH[b] | EC[b] (mS $cm^{-1}$) | RAS |
|---|---|---|---|---|---|
| 1 | 7.6 ± 0.4 a | 0.459 ± 0.09 c | 8.08 ± 0.04 a | 0.414 ± 0.04 d | 0.22 c |
| 2 | 7.5 ± 0.3 a | 0.899 ± 0.29 c | 7.81 ± 0.08 a | 1.151 ± 0.15 c | 0.25 c |
| 3 | 7.5 ± 0.4 a | 1.544 ± 0.24 b | 7.87 ± 0.07 a | 1.688 ± 0.30 b | 0.85 b |
| 4 | 7.8 ± 0.4 a | 2.571 ± 0.38 a | 8.20 ± 0.31 a | 2.826 ± 0.23 a | 3.83 a |
| 5 | 6.1 ± 0.2 b | 2.291 ± 0.43 a | 6.08 ± 0.12 c | 2.653 ± 0.04 a | 0.26 c |
| 6 | 6.6 ± 0.2 b | 2.746 ± 0.31 a | 6.55 ± 0.14 b | 3.040 ± 0.09 a | 0.67 b |
| Significance | ** | ** | ** | ** | ** |

Means with same letter (s) are not significantly different at $p < 0.05$, * Significant at $p < 0.00.5$, *** significant at $p < 0.01$ NS: not significant.

[a] pH and CE date February 20, 2021 (winter).

[b] pH and CE date May 31, 2021 (spring).

**Fig. 1.** Seed germination in the different treatments. The pots were placed in the order of the treatments for photography.

Substrates of all treatments were analyzed 15 weeks after sowing by extracting the soil profile (Table 3). The alfalfa in all pots were cut at a height of 5 cm above the ground. Plants cut from the same pot, together with the rest of the replicates of the same treatment, were stored until they were dried, and they were then transferred to the laboratory for analysis. All plant tissues were dried at 75 °C to a constant weight to determine their dry weight (DW) for each treatment.

### 2.3. *Statistical analysis*

The trial was performed using a completely randomized design with three replicates per treatment and the entire replication was rotated to minimize the impact of environmental variables within the greenhouse. A one-way ANOVA was used to test for differences in the following variables recorded during the crop cycle: pH and CE of soil; Ca, Mg, K and Na of substrate.

Growth parameters (height, stems and leaves) recorded during the crop cycle were analyzed by a two-way ANOVA in which treatments and week were included as factors. Differences were considered significant at $p < 0.05$ and when statistically significant effects were detected Tukey's multiple range test was applied to separate mean values. Growth parameters exhibiting statistically significant differences in the interaction between treatment and week were visually represented in graphical form. Statistical Package for Social Sciences (SPSS) v27.0 software (IBM SPSS Inc., Chicago, IL, USA) was used for all statistical calculations and graphics.

## 3. Results and discussion

### 3.1. *Seed germination*

Seed germination is one of the most important phases in the plant life cycle and it is normally limited by increasing strength of abiotic stresses, such as high salinity and drought [46]. Furthermore, seed germination is easily affected by the toxicity of soil contaminated with toxic elements [47]. The initial trial design had 10 treatments, although only alfalfa seeds from the first 6 treatments germinated, thus only these 6 treatments could be used in the data treatment (Fig. 1). Applying high amounts of PG (300 g $kg^{-1}$ PG) to soil may negatively impact alfalfa seed germination. In this case, the high levels of PG in the soil may have had a toxic effect on the alfalfa seeds, preventing them from germinating or hampering their growth and development. It is important to carefully consider the potential effects of soil amendments, including PG, on seed germination and plant growth [48].

The use of RM causes a significant increase in Na, as occurred with the use of fresh beet vinasse in the study of Tejada et al. [49], who observed that the physical, chemical and biological properties of the soil deteriorated despite the high organic matter content in the soil, possibly due to the high monovalent $Na^+$ cation content. High doses of PG and RM could have inhibited seed germination in some treatments, thus the amount of PG added in the pots where no alfalfa germination occurred was 300 g $kg^{-1}$ in treatments 8, 9 and 10. In addition, the substrate of treatment 7 (2550 g soil + 50 g $kg^{-1}$ PG + 100 g $kg^{-1}$ RM) also affected seed germination, possibly due

**Table 4**
ANOVA substrate Ca, Mg, K and Na recorded during the crop cycle.

| Treatments (T) | N[a] | P[a] | K | Ca | Mg | Na |
|---|---|---|---|---|---|---|
| | % | mg kg$^{-1}$ | cmol$_c$ kg$^{-1}$ | cmol$_c$ kg$^{-1}$ | cmol$_c$ kg$^{-1}$ | cmol$_c$ kg$^{-1}$ |
| 1 | 5.03 ±Nm | 16.40 ± 2.19 c | 0.75 ± 0.03 | 29.00 ± 1.24 c | 2.21 ± 0.09 abc | 0.54 ± 0.01 c |
| 2 | 5.02 ± Nm | 71.93 ± 11.41 c | 0.79 ± 0.07 | 31.23 ± 1.35 c | 2.44 ± 0.12 ab | 0.63 ± 0.02 c |
| 3 | 4.82± Nm | 80.46 ± 17.96 c | 0.76 ± 0.03 | 33.88 ± 1.80 c | 2.30 ± 0.08 abc | 2.24 ± 0.28 bc |
| 4 | 5.06 ±Nm | 69.82 ± 21.95 c | 0.78 ± 0.09 | 33.90 ± 3.33 c | 1.96 ± 0.18 c | 10.11 ± 1.66 a |
| 5 | 4.86 ±Nm | 800 ± 129 a | 0.86 ± 0.12 | 88.27 ± 5.75 b | 2.57 ± 0.18 a | 1.10 ± 0.06 c |
| 6 | 4.89 ±nM | 610.98 ± 82.15 b | 0.78 ± 0.05 | 111.79 ± 19.39 a | 2.18 ± 0.10 bc | 3.18 ± 0.22 b |
| Significance | ns | ** | ns | ** | ** | ** |

Means with same letter (s) are not significantly different at $p < 0.05$, * Significant at $p < 0.00.5$, ** significant at $p < 0.01$, NS: not significant. Nm: not measured.

[a] Total N determination though Dumas Method. P available extraction: Olsen, S.R., Cole, C.V., Watanabe, F.S. y Dean, L.A. (1954).

to the high amount of RM (100 g kg$^{-1}$). Plants can be exposed to different environmental stresses during their growth stages. In this sense, Zhang et al. [50] found that higher concentrations of iron nanoparticles FeNPs (50–200 mg L$^{-1}$) inhibited the growth of alfalfa seedlings. Salinity is amongst the most severe stressors, with adverse effects on the germination and growth of plants. To study the effects of different levels of salt concentrations on seed germination, many experiments have been conducted under laboratory and field conditions [51]. Wang et al. [46] studied the differential responses of six alfalfa cultivars to salt and drought stresses during germination by analyzing the germination rate under stresses corresponding to different NaCl concentrations; authors selected some alfalfa varieties as stress-tolerant and stress-sensitive cultivars for further characterization. Wang et al. [46] reported that transgenic alfalfa with enhanced stress tolerance could be useful for sustainable agriculture in marginal soils, including desertified areas and alkalinized soils.

### *3.2. pH and EC for the substrate combinations*

The pH of the substrate can also affect the availability of nutrients to plants, as some nutrients are more readily available at certain pH ranges. The pH and EC (dS cm$^{-1}$) of the drainage were analyzed at midterm and at the end of the study for all treatments (Table 3). Significant effects of treatments were found on pH and EC. The pH varied among the prepared substrate combinations both halfway through and at the end of the cultivation, changing between 6.1 and 7.6 halfway through the trial. In the treatments with the highest PG content (5%), the drainages were most acidic. This suggests that the presence of high levels of PG in soil can lead to the production of more acidic drainage, mainly due to the remaining phosphoric acid trapped between the PG particles after the industrial process [52]. Treatments 5 and 6 showed a similar behavior with pH values below 7. In addition, the pH of this group became more acidic at the end of the study: T5 from 6.1 to 6.08 and T6 from 6.6 to 6.55. It is important to carefully consider the potential impacts of PG on the acidity of drainage, as acidic drainage can have negative environmental impacts, such as soil and water contamination, if they are released into the environment. It may also be necessary to implement measures to neutralize the acidity of drainages containing high levels of PG to minimize these potential impacts. Nevertheless, alfalfa plants are known to have strong root systems capable of effectively taking up nutrients and other substances from the soil [53]. This ability to absorb nutrients and other substances from the soil can make alfalfa plants particularly useful for remediation of contaminated sites, as they are able to effectively remove potentially toxic elements from the soil. However, it is important to consider the potential impacts of using alfalfa for remediation, as the plants may also take up and accumulate harmful substances that can be toxic to humans or animals if ingested. Additionally, the effectiveness of alfalfa for remediation may depend on the specific contaminants present in the soil and the specific conditions at the contaminated site.

Treatments 1, 2, 3 and 4 formed the same group with values above 7. As was the case halfway through the investigation, treatments 1, 2, 3 and 4 behaved in a similar manner under pH values ranging from 7.8 for treatment 2 to 8.2 for Treatment 4. In addition, the pH of this group became more alkaline at the end of the investigation: T1 from 7.6 to 8.1, T2 from 7.5 to 8.8, T3 from 7.5 to 7.9, and T4 from 7.8 to 8.2. On the other hand, at the end of the investigation, the most acidic pH was generated in treatment 5, with a value of 6.1. It is important to highlight that the pH was also the highest with the same value in the month of February (Table 3).

RM is a highly alkaline material, with a pH value that can range from 11 to 13.5. As a result, it can be used to increase the pH of soil, which can be beneficial for certain plants that prefer more alkaline conditions, in addition to its pH-adjusting properties [54]. Similarly, the EC values of substrate combinations varied between winter (February) and spring (May) (i.e., crop cycle). EC values increased with increasing PG and RM contents in both winter and spring. The initial EC value of the PG-based substrates ranged from 0.89 to 2.7 mS cm$^{-1}$ compared to the control value of 0.46 mS cm$^{-1}$. Treatments 4, 5 and 6 behaved similarly, with values above 2.5 mS cm$^{-1}$ halfway through and at the end of the cultivation. The highest substrate EC values were observed for T6 halfway through and at the end of the cultivation. The addition of PG and RM to soil increased the EC of the soil, which is a measure of the amount of salts present in the soil. When the EC of soil increases, it can indicate an increase in the concentration of salts in the soil, including both beneficial and potentially harmful salts [55]. This increase in salts can have several impacts on plants, including changes in the uptake of nutrients, the ability of plants to absorb water, and the overall health and growth of the plants. In particular, the presence of high levels of salts in the rhizosphere zone (the area of soil surrounding plant roots) can affect the plants' ability to absorb water and

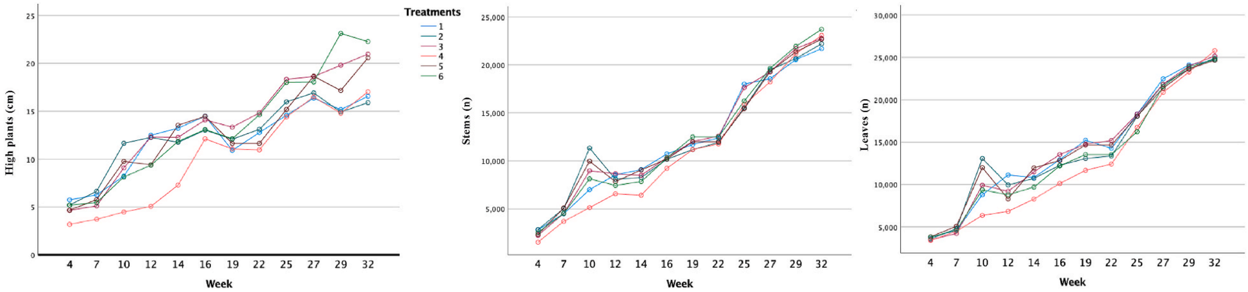

**Fig. 2.** Growth parameters: height (cm), stems and leaves (numbers) recorded during the crop cycle.

nutrients, which can impact their growth and development. It is important to carefully consider the potential impacts of increased salt levels in soil and to manage the levels of PG and RM applied to soil to minimize potential negative effects on plants.

All treatments, except for the control treatment (T1), showed an increase in EC obtained halfway through and at the end of the cultivation (Table 3).

### *3.3. Influence of substrates on plant tissue mineral content*

The type of substrate used can have a significant influence on the levels of nutrients and other elements in the soil, which can impact the growth and development of plants. It is important to carefully consider the potential impacts of different substrates on the nutrient levels in soil and to select the most appropriate substrate based on the specific needs of the plants being grown. In this study, the content of N and K did not show significant differences between treatments. However, T3 showed the lowest N value (4.8%) and T4 showed the highest N value (5.1%). On the other hand, the control treatment (T1) showed the lowest K value (0.75 $\mathrm{cmol_c\ kg^{-1}}$) and T5 obtained the highest K value (0.86 $\mathrm{cmol_c\ kg^{-1}}$) (Table 4).

Significant differences in P, Ca, Mg and Na were observed in the substrates throughout the crop cycle in relation to treatment. The results showed that, in the substrates, the highest concentrations of P (800 mg $\mathrm{kg^{-1}}$) and Mg (2.57 cmolc $\mathrm{kg^{-1}}$) were observed in T5 (Table 4) and the Na concentration was higher (10.11 cmolc $\mathrm{kg^{-1}}$) in T4 than in the rest of the treatments, which is due to the high RM content in T4 (10%), being the highest percentage of RM among all the substrates used. In addition, treatments 1, 2, 3 and 5 formed the same group, with mean Na values below 2.25 $\mathrm{cmol_c\ kg^{-1}}$. The substrates that were mixed with RM (T3, T4 and T6) obtained the highest Na values (2.24 $\mathrm{cmol_c\ kg^{-1}}$, 10.11 $\mathrm{cmol_c\ kg^{-1}}$ and 3.18 $\mathrm{cmol_c\ kg^{-1}}$, respectively). The use of RM caused a significant increase in Na, as occurred with the use of fresh beet vinasse in Tejada et al. [49], who observed that the physical, chemical and biological properties of the soil deteriorated despite the high organic matter content in the soil, possibly due to the high content of monovalent $Na^+$ cation.

The contribution of Ca to the soil through the amendment with PG can influence the absorption of other elements, either enhancing or inhibiting it, which could lead to deficiencies of certain trace elements, or to the increase in plant concentrations of some heavy metals [56]. The Ca value was highest in T6 (111.79 cmolc $\mathrm{kg^{-1}}$), followed by T5, thus treatments with a higher PG content showed the highest Ca inputs, as can be expected. The Ca content of PG is relatively high, and it can be a source of this essential plant nutrient.

The long-term application of PG to soil can have a range of beneficial effects on physiological and biochemical processes in plants. These effects include reducing soil acidity, increasing the availability of important nutrients like phosphorus (P), calcium ($Ca^{2+}$), magnesium ($Mg^{2+}$), and sulfur ($SO_2^{\,4}$ –S), and improving plant nutrition. PG can also improve the overall health of the plant and lead to higher biomass yield (or the amount of plant material produced). These effects can be particularly beneficial for plants growing in soil with low fertility or high levels of stress, as PG can help to improve soil quality and support healthier plant growth [21]. RM also contains a range of plant nutrients, including silicon, iron, and aluminum, which can support plant growth. Regarding biomass, some authors suggest that the treatments studied can increase alfalfa biomass [50], while others report no significant differences in biomass per plant among different treatments [57].

### *3.4. Time-evolution of the alfalfa growth parameters*

Table 4 shows the alfalfa plant growth parameters measured throughout the crop cycle, up to the first and only cutting of the plants. Analyses of the data showed that the week effects, treatments and the interaction of treatments and week were significant, and the results were consistent across the week. The interaction between the two factors (treatment and week) on the response of height (cm) means that the effect of the treatments on the height of alfalfa depends on the week. This is evident in the graph, as the lines cross, indicating that the effect of the treatments varies depending on the week. For example, in the case of T4, the mean values of height (9.22 cm) are lower than those of T3, T5 and T6 (12.78 cm, 11.87 cm and 12.61 cm, respectively). Height growth showed the typical continuous length pattern in all treatments, including the control (Table 4). In all cases, the highest growth rate was observed between weeks 29 and 32 of cultivation. The maximum height reached by the control plants was 11.4 cm, while in the different treatments this value ranged between 9.22 and 12.78 cm. The application of PG + RM in T3 improved plant height (12.78 cm) and the number of stems (12.43) as compared with the control treatment. Except for treatment T4, the mixtures of PG and RM used in the other treatments can be considered sufficient to produce plants of adequate height. Stem number in the different treatments showed significant

**Table 5**
Growth parameters recorded during the crop cycle.

| Treatments (T) | Height (cm) | Stems[a] | Leaves[a] |
|---|---|---|---|
| 1 | 11.40 ± 3.89 bc | 12.13 ± 6.14 a | 14.261 ± 6.93 a |
| 2 | 11.62 ± 3.86 bc | 12.22 ± 5.97 a | 14.10 ± 6.75 a |
| 3 | 12.78 ± 5.32 a | 12.43 ± 6.44 a | 14.26 ± 7.06 a |
| 4 | 9.22 ± 4.81 d | 11.15 ± 6.86 b | 12.53 ± 7.35 b |
| 5 | 11.87 ± 4.62 abc | 12.27 ± 6.16 a | 14.26 ± 6.62 a |
| 6 | 12.61 ± 6.04 ab | 12.27 ± 6.65 a | 13.50 ± 6.81 a |
| Significance | ** | ** | ** |
| **Week (W)** | | | |
| 4 | 4.77 ± 1.05 h | 2.38 ± 0.61 i | 3.66 ± 0.32 h |
| 7 | 5.49 ± 1.34 h | 4.57 ± 0.63 h | 4.64 ± 0.39 h |
| 10 | 8.56 ± 2.41 fg | 8.42 ± 2.21 g | 9.92 ± 2.42 fg |
| 12 | 10.15 ± 3.18 ef | 7.86 ± 0.88 g | 9.03 ± 1.73 g |
| 14 | 11.65 ± 2.59 de | 8.19 ± 1.32 g | 10.50 ± 2.40 f |
| 16 | 13.54 ± 1.53 c | 10.16 ± 0.70 f | 12.32 ± 2.02 e |
| 19 | 11.86 ± 1.37 cd | 11.76 ± 0.87 e | 13.82 ± 1.70 d |
| 22 | 12.99 ± 1.94 cd | 12.19 ± 0.64 e | 13.91 ± 1.57 d |
| 25 | 16.09 ± 2.10 b | 16.45 ± 1.13 d | 17.61 ± 1.11 c |
| 27 | 17.52 ± 1.14 g | 19.08 ± 0.80 c | 21.66 ± 0.88 b |
| 29 | 17.50 ± 3.55 ab | 21.23 ± 0.58 b | 23.74 ± 0.67 a |
| 32 | 18.89 ± 3.63 a | 22.68 ± 0.78 a | 25.00 ± 0.66 a |
| Significance | ** | ** | ** |
| Interaction TxW | ** | ** | * |

Means with same letter (s) are not significantly different at $p < 0.05$, * Significant at $p < 0.00.5$, ** significant at $p < 0.01$, NS: not significant.

[a] Number stems and leaves.

differences (p ≤ 0.05). Treatment 4 behaved differently from the rest of the treatments for the number of stems and the number of leaves, with values below 12 and 13, respectively.

The time effect was also significant, as the significance difference for plant height, and number of stems and leaves (Fig. 2). The alfalfa plants performed as expected over time, showing sustained growth in plant height, number of stems and number of leaves.

### *3.5. Determinations of alfalfa dry matter at the end of the trial*

Data related to the influence of PG and RM application on dry matter yield of alfalfa and uptake of macro and micronutrients are presented in Table 5. Treatment 4 showed the highest Ca and Fe values (2.44 g $kg^{-1}$ and 138.8 mg $kg^{-1}$, respectively). In addition, the plants in T2 assimilated the least amount of Fe, with a mean value of 53.8 mg $kg^{-1}$, and the highest amount of Mg, with a mean value of 3.07 g $kg^{-1}$. On the other hand, plants in treatment 3 had the lowest Ca, Mg, Mn and P uptake, with mean values of 1.17 g $kg^{-1}$, 2.06 g $kg^{-1}$, 2.73 g $kg^{-1}$ and 13.8 mg $kg^{-1}$, respectively. The control treatment (T1) showed the highest values for Cu, S and Zn, being 10.96 mg $kg^{-1}$ for Cu, 3.31 g $kg^{-1}$ for S, and 45.0 mg $kg^{-1}$ for Zn. However, the plants in T5 assimilated the least amount of Cu, S and Zn, with mean values of 6.92 g $kg^{-1}$, 2.39 g $kg^{-1}$ and 24.2 mg $kg^{-1}$, respectively.

PG application improved the uptake of P in alfalfa. The substrates containing the highest amount of PG obtained the highest P values, with the mean values in T5 and T6 being 4.79 g $kg^{-1}$ and 5.07 g $kg^{-1}$, respectively. Furthermore, the alfalfa plants in T6 assimilated the highest amount of K, with a mean value of 37.5 g $kg^{-1}$. Treatment 4 showed the lowest mean value of K, with 30.9 g $kg^{-1}$ (Table 6).

**Table 6**
Mean values chemical characteristics of the alfalfa recorded at the end of the crop cycle (mg $kg^{-1}$).

| Treatments | Ca[1] | Cu | Fe | K | Mg | Mn | P | S | Zn |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 17517.50 | 10.96 | 100.72 | 36081.28 | 2645.95 | 18.26 | 3310.15 | 3421.02 | 45.04 |
| 2 | 23019.18 | 7.58 | 53.80 | 33464.54 | 3069.01 | 19.70 | 2880.34 | 3115.23 | 26.52 |
| 3 | 11710.81 | 7.73 | 90.27 | 30909.64 | 2065.73 | 13.82 | 2728.14 | 2721.64 | 24.80 |
| 4 | 24428.27 | 8.35 | 138.76 | 30893.51 | 2855.48 | 27.61 | 2915.78 | 3138.08 | 34.51 |
| 5 | 14647.32 | 6.92 | 92.22 | 31034.91 | 2298.92 | 151.71 | 4729.01 | 2397.12 | 24.24 |
| 6 | 16292.12 | 7.85 | 126.34 | 37554.09 | 2787.06 | 73.26 | 5071.23 | 3128.34 | 26.17 |

## 4. Conclusion

The study found that high levels of phosphogypsum (PG) in soil can be toxic to alfalfa seeds and hamper their growth and development, but the application of PG and red mud (RM) to soil can increase the availability of certain nutrients for alfalfa, suggesting that waste materials like PG and RM could be used as a valuable resource for plant nutrition.

Germination is a critical stage in the life cycle of a plant, and it can be sensitive to soil contaminated with toxic substances. In this case, the high levels of PG in the soil had a toxic effect on the alfalfa seeds, preventing them from germinating or hampering their growth and development. It is crucial to consider the potential impact of soil amendments such as PG on both seed germination and plant growth. The reported results showed that, as salinity increased, the percentage of germination decreased. Applying high amounts of PG to soil (300 g $kg^{-1}$ PG) will negatively affect alfalfa seed germination. In addition, the use of RM causes a significant increase in Na.

Giving due consideration to the potential effects of PG on drainage acidity is essential, since acidic drainage, if released into the environment, can cause harmful environmental consequences, such as soil and water pollution, and measures to neutralize the acidity of drainages containing high levels of PG may also be necessary to minimize these potential impacts. The addition of PG and RM to soil can increase the electrical conductivity of the soil. The presence of high levels of salts due to PG and RM inputs in the rhizosphere zone (the area of soil surrounding plant roots) can affect the ability of plants to absorb water and nutrients, which can impact their growth and development. Our results may be the first to show the feasibility of using PG as input for plant nutrition, since, when applying higher doses (50 g of PG per kg of soil), the concentration of Ca rises. PG application improved the uptake of P in alfalfa. The application of PG and RM to the soil increased the availability of important nutrients for alfalfa, such as phosphorus (P), calcium ($Ca^{2+}$) and magnesium ($Mg^{2+}$). However, further research is necessary to optimise the doses of waste (PG and RM) for each crop, such as alfalfa growing systems in this case.

The European Union has identified and documented certain natural resources as critical raw materials (CRM) to prevent their scarcity, and phosphate is one of these. Thus, the buried PG ponds may be used in the future as an agricultural supply of elements such as P and Ca.

## CRediT authorship contribution statement

**Pedro Palencia:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **José Luis Guerrero:** Writing – review & editing, Writing – original draft, Validation. **Rebeca Millán:** Formal analysis, Data curation. **Fernando Mosqueda:** Resources, Project administration, Funding acquisition. **Juan Pedro Bolívar:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

## Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Juan Pedro Bolivar reports administrative support was provided by University of Huelva. Bolivar reports a relationship with University of Huelva that includes: employment. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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