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# Limestone Quarry Waste Promotes the Growth of Two Native Woody Angiosperms

*Muhammad Umar Hayyat[1], Zafar Siddiq[2]*, Rashid Mahmood[1], Amin U. Khan[1] and Kun-Fang Cao[3]**

*[1] Sustainable Development Study Centre, GC University Lahore, Lahore, Pakistan, [2] Department of Botany, GC University Lahore, Lahore, Pakistan, [3] Plant Ecophysiology and Evolution Group, State Key Laboratory for Conservation and Utilization of Subtropical Agro-Bioresources, Guangxi Key Laboratory of Forest Ecology and Conservation, and College of Forestry, Guangxi University, Nanning, China*

Limestone quarrying is an active mining practices generating bulk of solid remains and altering the habitat by the removal of plants; however, the utilization of such waste for the growth of plants has not been investigated much. The present study aimed to evaluate the effects of limestone quarry waste on the growth of two native plants by analyzing its physicochemical properties and utility for plantation purposes, while determining whether mitigation measures would be required for the habitat restoration of quarry site. Two species, *Acacia modesta* and *Adhatoda vasica* were selected from the quarry site habitat. These plants were grown in different proportions of quarry waste, and garden soil was used as a control. Growth was assessed by recording plant height, number of branches per plant, root and shoot length, and total biomass. We also analyzed the N, P, K, Na, Ca, and Mg contents of the root and shoot tissues of both species. We found a significant increase in plant height (1.24- and 1.19-fold greater than controls for *A. modesta* and *A. vasica*, respectively). Differences in the number branches, root, shoot length, and biomass were also found. A significant and positive relationship was found between the mineral content in roots and the total plant biomass across both species. We conclude that (1) the mining solid waste contained the necessary minerals for the studied plant species and no amelioration would be required for restoration of such sites with the selected indigenous plants; and (2) the quarry waste promoted the growth of the two selected species. The results of the present study can be used to plan habitat restoration in limestone mining areas that have lost plant cover.

**Keywords: *Acacia modesta*, calcium, mining, habitat restoration, biomass, mineral uptake**

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*Sun Yat-sen University, China*

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*Rodolfo Gentili,*
*University of Milano-Bicocca, Italy*
*Wenqi Luo,*
*Sun Yat-sen University, China*

***Correspondence:**
*Zafar Siddiq*
*zafareco@outlook.com*
*Kun-Fang Cao*
*kunfangcao@gxu.edu.cn*

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## INTRODUCTION

Mineral deposits are finite and will eventually be exhausted (Jowitt et al., 2020). Mining of minerals is a temporary land use change that alters the original soil strata and produces large amounts of soil remains (Johnson et al., 1994; Basommi et al., 2016). The increasing demand for minerals is causing expansions in mining areas; at present, 57,277 km$^2$ is being used for mining operations on a global scale (Maus et al., 2020). In many mining operations, waste production, and its disposal can cause extensive and long-lasting disturbances on the natural ecological processes such as changes in species composition, primarily due to unstable substrates for plant growth (Ballesteros et al., 2014; Virah-Sawmy et al., 2014; Reta et al., 2018; Sonter et al., 2018). On a large scale, mining operations impact both the abiotic and biotic components of particular ecosystems (Hester et al., 1994; Millennium Ecosystem Assessment, 2005). The abiotic impacts include changes in soil profile, nutrients, texture, and other physicochemical properties such as pH electrical conductivity and production of particulate matter due to crushing of rocks. The assessment of the physicochemical properties of solid waste is essential to understand its fertility status. Khan et al. (2020) and Okereafor et al. (2020) found that some mining operations can release toxic elements such as arsenic, lead, and nickel, which can pollute the soil and water. Mining can impact on ecology through habitat loss and fragmentation (Gajic et al., 2018). Ntshane and Gambiza (2016) reported 20–30% habitat loss due to mining in South Africa. Sonter et al. (2015) found that a significant amount of carbon emissions ascribed to deforestation was linked with charcoal mining. Similarly, mining in Indonesia resulted in a sixfold increase in habitat loss (Paull et al., 2006).

The utilization of large amounts of mining waste for establishing vegetation in mining areas depends on its physicochemical properties. As a general rule, only a few stress-tolerant plant species are able to grow in postmining substrates (Zhang et al., 2017). This is mainly due to high concentration of minerals, scarce availability of organic matter, and moisture and disruption of soil strata (Zeleznik and Skousen, 1996; Gorman et al., 2001; Grant and Koch, 2007; Skousen and Zipper, 2014). Furthermore, the mining areas can also be inhabited by some exotic species, such as Monty et al. (2019), who recorded the significant invasion of alien plants on limestone quarry wastes, which resulted in indigenous species habitat loss. Additionally, limestone quarries can cause soil erosion and dereliction of mined sites (Langer, 2001). A rapid solution for avoiding soil erosion and plant invasion is the plantation of native species for rehabilitating postmining landscapes. Native plant species can repopulate these postmining landscapes and eventually restore native vegetation.

The social and legislative context in many parts of the modern world means that some form of postmining land use and natural ecosystem reestablishment goals will be set before the permission is granted for a new mine (Skousen et al., 1994; Skousen and Zipper, 2014). Species reestablishment and sustainability are the major factors that determine the success of ecological restoration (Rodríguez-Seijo and Andrade, 2017; Li et al., 2018; Song, 2018). Species selection for restoration can be based on the chemical analysis of plant tissues, which can be indicative of species capacities to uptake the minerals in the solid waste of mining sites. Species more apt at mineral uptake can be effective in site restoration and more easily established (Marcus et al., 2018) such plant-tissue analyses that can be helpful in predicting plant growth. Minerals in mining waste can have positive or negative impacts on plant growth, e.g., plant height and branch sprouting (Jim, 2001; Gajic et al., 2018).

Restoration actions should aim to reestablish ecosystem services through species diversity and survival (Montoya et al., 2012). Appropriate measures, such as the possible solid waste amelioration with fertile soil and the selection of suitable species for mineral uptake, should be taken for the utilization of mining solid waste to revegetate the mining site. These cannot only reduce the impacts of mining on such transformed habitats but also increase the population sizes of the selected species (Macdonald et al., 2015; Gentili et al., 2020). Such species are suitable for ground stabilization and to green a barren anew (Bengson, 1995; Yirdaw and Luukkanen, 2003). Furthermore, being adaptive to local environmental and edaphic conditions, these native species can be established as self-sustaining plant communities. Therefore, the use of native plant species for landscape restoration in mining areas or to replace exotic plants can help to reverse the trend of species loss (Richards et al., 1998; Román et al., 2015).

Pakistan has extensive deposits of limestone in the provinces of Punjab, Sindh, Balochistan, and northern areas. These deposits are located in geological formations that range in age from the pre-Cambrian to the Eocene (Hamid et al., 2012). The presence of these deposits attracts mining companies, resulting in accelerated habitat change for the indigenous plants (Drewes et al., 2007). In the limestone quarry, *Acacia modesta* forms a bi-species climatic climax community with *Olea cuspidata*, while *Adhatoda vasica* and *Dodonea viscosa* both are understory shrubs (Champion et al., 1965). All of these species are valuable sources of livelihood for the local community as they are a source of traditional medicines, fodder, and animal grazing and control soil erosion (Khan, 2013; Khan et al., 2019). Considering the ongoing acceleration of mining impact on natural vegetation, there is a need to utilize the mining solid waste. As the quarry sites with waste heaps become barren, revegetation may begin by planting more productive species such as herbs and shrubs of the area, capable of using the existing postmining solid waste (Swanson et al., 2010). The present study was designed with the following objectives:

1. To analyze the physicochemical properties of solid waste from the limestone quarry.
2. To assess the effects of this waste on the growth of selected plant species.

As the restoration of an abandoned site with limestone waste may follow the natural succession, we hypothesized that the shrub *A. vasica* being the early colonizer may accumulate more minerals in its tissues than *A. modesta*.

## MATERIALS AND METHODS

### Study Site and Quarry Waste

The study was carried out in the limestone quarry of Tobar, in the Jhelum District of Pakistan, located in the salt range (**Figure 1**). This site is 9 km away from the chemical production facility of a multinational company, where limestone is one of the raw materials used in the manufacturing process. The quarrying operation produces a large amount of solid waste at a rate of 88.18–132 tons/day (Hayyat, 2008). Solid waste from limestone quarrying has a variety of uses, ranging from the manufacturing of

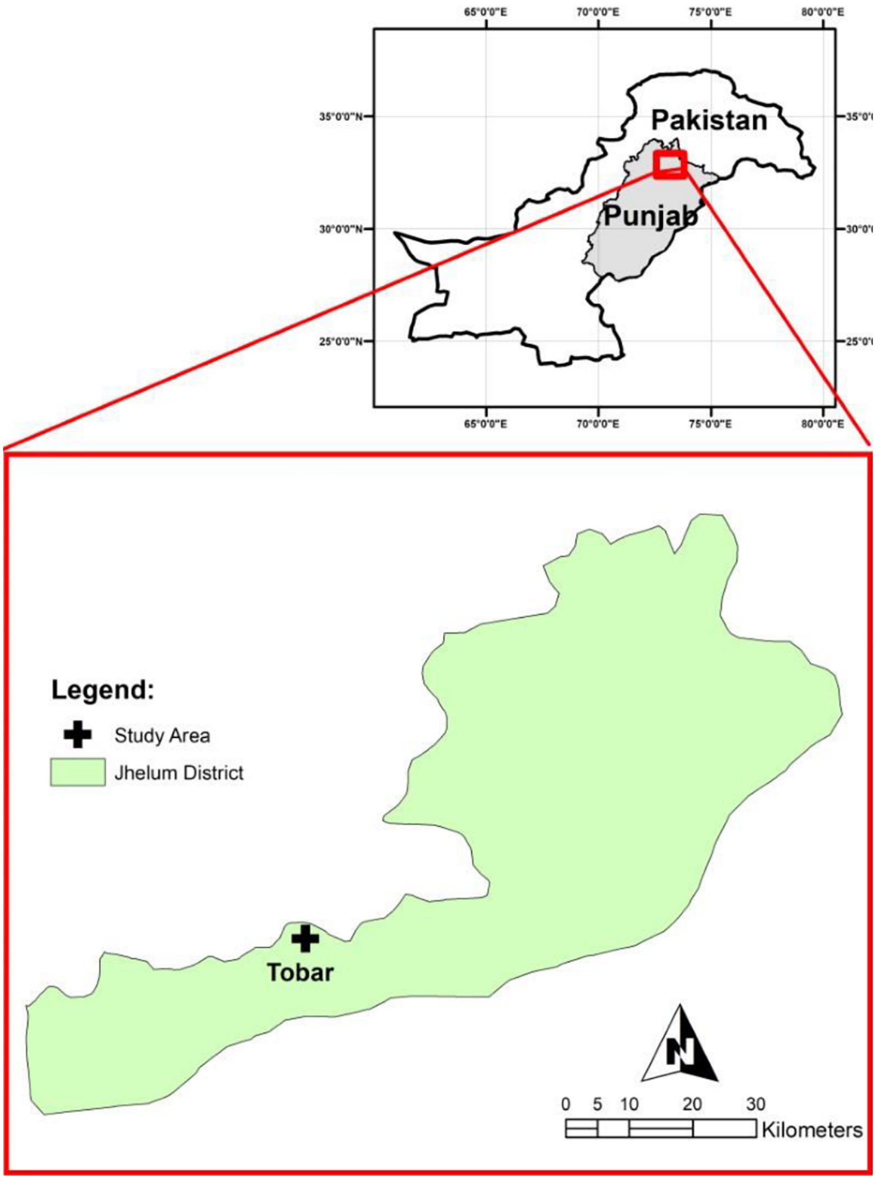

**FIGURE 1 |** The location of study site in the Tobar area, Punjab, Pakistan.

masonry blocks to cement production (Felekoglu, 2007; Turgut, 2008). In Pakistan, limestone is cheap and its transport expensive; therefore, the use of limestone quarry waste is limited. The average annual production of limestone is 9.95 tons in Pakistan. Mining has a significant potential for the employment of local people, but the related policies are to be redesigned for sustainability (Government of Pakistan, 2004; Ali and Rehman, 2020).

## Waste Material

Solid waste was collected from the mining quarry sites located in Tobar, Punjab, Pakistan (**Figures 2a,b**). Ten different 30-kg samples of waste were collected randomly from different locations of the waste heap, avoiding particle size >3.0 mm. The samples were mixed to form a composite sample, which was then used for physicochemical analyses.

## Physicochemical Analysis of Soil and Plant Tissues

In the laboratory, solid waste samples were crushed in a grinding mill. The particle size of solid waste from the quarry was determined by sieve, passing 0.25–2.25-mm-diameter particle sizes (McLean and Ivimey-Cook, 1952). To ensure the availability of solid waste in the treatments, the material retained on the sieve was reground until the entire sample passed through the sieve. The textural class of the soil was

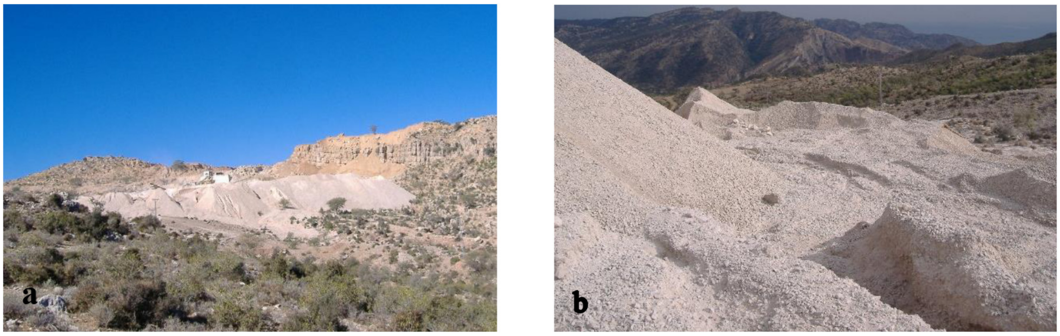

**FIGURE 2 |** Mining site broader view **(a)**; close view of the waste heaps **(b)**.

determined using a hydrometer according to the method described by Bouyoucos (1962). Garden soil was used to prepare different concentrations of solid quarry waste for the various treatments. A soil:water suspension (1:1) was prepared (He et al., 2012) to determine the pH using a pH meter (inoLab, Germany). A soil saturation extract was prepared for further analyses. The electrical conductivity was measured using an EC meter (SEnsoDirect Con 200, Netherlands). The nitrogen (N) percentage in the samples was determined according to Jones (1991) using the Kjeldahl method. To estimate the phosphorus content (P), a UV-VIS spectrophotometer (SpectroScan 80 D, Cyprus) was used to measure absorbance at 410 nm, as described by Olsen and Sommers (1982). Calcium (Ca) and magnesium (Mg) were determined by titration, while sodium (Na) and potassium (K) were quantified using flame photometry (AFP 100, United Kingdom).

## Experimental Setup

The experiment was carried out in ceramic pots (30 cm in diameter) filled with various garden soil/solid waste mixtures, prepared at the Botanic Garden, GC University, Lahore, Pakistan (**Table 1**). Five treatments for each species and five replicates of each treatment were prepared. Seeds of *A. modesta* and cuttings of *A. vasica* were collected from the study area and raised at the nursery at the Botanic Garden, GC University. Eight-week old plants of uniform height 24 cm (±0.47) for *A. modesta* and 15 cm (±0.79) for *A. vasica* were used for the experiment. One plant of each species was grown in each pot and growth data, that is, plant height (cm) and number of branches per plant were measured every 15 days and harvested after 165 days.

**TABLE 1 |** Preparation of various waste/soil mixtures for treatments.

| Sr. | Treatment | Concentrations | Solid waste | Garden soil |
|---|---|---|---|---|
| 1. | $T_0$ | Control | 00 | 100 |
| 2. | $T_1$ | 25% | 25 | 75 |
| 3. | $T_2$ | 50% | 50 | 50 |
| 4. | $T_3$ | 75% | 75 | 25 |
| 5. | $T_4$ | 100% | 100 | 00 |

## Plants Harvesting and Biomass Assessment

After 165 days, all the plants from both species were carefully extracted from the pots, relieved from excessive soil, placed in labeled zipper bags, and brought to the laboratory for further analyses. The parameters recorded for each plant included the following: plant height (cm), number of branches, root length (cm), and dry weight (g) of above- and belowground parts. To obtain the dry weight, the plants were oven-dried to a constant weight at 78°C. Before chemical analyses, plant materials were passed through a stainless-steel grinder with a 20-mesh sieve, and then mixed thoroughly. Mass-based chemical analysis of plant shoot and root tissues was conducted to determine N, P, Na, P, Ca, and Mg contents, as described in the "Physicochemical Analysis of Soil and Plant Tissues" section mentioned above.

## Statistical Analysis

Treatment means, standard errors, and least significant differences were calculated for various parameters. One-way analysis of variance (ANOVA) was conducted using the software package COSTAT, version 3.03, to determine the significance of differences among treatment means $P < 0.05$. Sigma Plot v.14

**TABLE 2 |** Physicochemical analyses of solid quarry waste and garden soil.

| Attributes | Solid quarry waste | Garden soil |
|---|---|---|
| Particle size | | Sand: 79.50% |
| $\geq 2.00$ mm | 44.75 ± 7.7% | Silt: 10.0% |
| $\geq 1.00$ mm | 10.65 ± 1.0% | Clay: 10.5% |
| $\geq 0.25$ mm | 10.65 ± 3.8% | |
| $\leq 0.25$ mm | 33.05 ± 12.7% | |
| Texture | Sandy-gravel | Loamy sand |
| pH | 8.16 ± 0.01 | 8.23 ± 0.00 |
| EC ($dSm^{-1}$) | 0.06 ± 0.00 | 0.01 ± 0.00 |
| N (%) | 1.53 ± 0.00 | 8.2 ± 0.05 |
| P (%) | 0.01 ± 0.00 | 0.01 ± 0.00 |
| K (ppm) | 2.95 ± 0.06 | 3.4 ± 0.1 |
| Na (ppm) | 3.25 ± 0.16 | 6.95 ± 0.15 |
| $Ca^{++}$ (%) | 2.37 ± 0.00 | 0.11 ± 0.00 |
| $Mg^{++}$ (%) | 0.02 ± 0.00 | 0.07 ± 0.00 |

was used to generate the graphics. SPSS v. 13 was used to analyze the effects of treatment and their different duration on the growth of both species. Plant growth was considered a response variable, while treatment and duration were considered factors.

# RESULTS

## Physicochemical Analysis of Solid Waste and Garden Soil

The physicochemical analysis of quarry waste and garden soil showed that the particle size of solid waste can be divided into four different classes i.e., $\geq$2.00, $\geq$1.00, $\geq$0.25, and $\leq$0.25 mm. In the quarry waste used in the present study, the most abundant class was the $\geq$2.00-mm class; 44.75 ± 7.77% of the quarry waste had this particle size. The second most abundant was the $\leq$0.25-mm-size class (33.05 ± 12.79%). There were almost equal percentages of particles in the $\geq$1.00- and $\geq$0.25-mm-size classes, but greater variations in the percentage of particles in the latter size class. The texture of the solid waste was sandy-gravel, while that of the garden soil was loamy-sand. The pH of the solid waste was slightly lower than that of the soil. The electrical conductivity of the solid waste was higher than that of garden soil. The soil contained higher levels of N, P, K, Mg, and Na than the solid waste. The solid waste had a higher Ca content than garden soil (**Table 2**).

## Effect of Quarry Waste on Plant Height and Number of Branches per Plant

After 165 days of growth, significant differences in the total plant height and number of branches per plant were measured among the different treatments for both species. The maximum height per plant was in $T_4$ (76.66 ± 1.85, 52.31 ± 1.73) and the minimum in $T_0$ (52.0 ± 0.91, 30.1 ± 1.06) for both *A. modesta* and *A. vasica*, respectively. Similarly, the maximum number of branches was also in $T_4$ (8.30 ± 1.17) and the minimum in $T_0$ (6.20 ± 0.47) for *A. modesta*. *A. vasica* did not exhibit significant differences in its branch number (**Figure 3**). The changes in the total plant height and number of branches for both species at intervals of 15 days are shown in **Supplementary Figures 1–4**. Furthermore, the mixed-model analysis showed that the solid waste treatment, number of days, and the interaction of solid waste treatment with the number of days had a significant effect on the total plant height for both species (**Table 3**).

## Effect of Quarry Waste on Aboveground, Belowground, and Total Biomass per Plant

There were significant differences in the aboveground biomass in all treatments from $T_1$ to $T_4$ compared with $T_0$. The lowest aboveground biomass was observed in $T_0$, while the highest aboveground biomass was observed in $T_4$. However, the belowground biomass of $T_1$ and $T_2$ was not significantly different from $T_0$, but a significant difference was observed between $T_3$ and $T_4$ as compared with $T_0$. The total biomass of *A. modesta* was significantly different from that of $T_1$ to $T_4$ in comparison with that of $T_0$ (**Figures 4A–C**). For *A. vasica*, the aboveground biomass was significantly greater in $T_4$ than in all other treatments. There were significant differences in the aboveground biomass between $T_0$ and $T_2$, $T_3$, and $T_4$. The belowground biomass was lowest in $T_0$ and highest in $T_4$. The root biomass tended to increase as the percentage of quarry waste in the soil matrix increased. However, significant differences were observed between $T_3$, $T_4$, and $T_0$. The total biomass of *A. vasica* was significantly greater in the $T_2$, $T_3$, and $T_4$ than $T_0$ (**Figures 4D–F**).

## Chemical Analyses of Shoot and Root Tissues of *A. modesta* and *A. vasica*

For both species, the highest nitrogen content was observed in $T_4$. There were significant differences among the different treatments in terms of mass-based N content percentage (%). Roots showed higher N content than shoots; this pattern was observed in all treatments. The highest P content was in $T_4$ and the lowest in $T_0$, with significant variations among the other treatments. The same pattern was observed for K (highest in $T_4$, lowest in $T_0$, with significant differences among others). Na content was highest in $T_0$ and lowest in $T_4$, and there were significant differences among the other treatments. In both roots and shoots, the highest Ca concentration was observed in $T_4$ and lowest in $T_0$, with significant differences among the others and a lower Ca content in the roots than in the shoots. The Mg concentration was highest in $T_4$ and lowest in $T_0$, but the difference between $T_4$ and $T_3$ was not significant. The magnesium concentration was lower in the roots than in the shoots in all treatments (**Tables 4**, **5**).

## Correlation of Minerals With Total Plant Biomass

In the studied treatments, a significant and positive relationship was found between the total biomass of *A. modesta* and *A. vasica*, respectively, with the N content ($R^2$ = 0.82 and 0.90), P ($R^2$ = 0.89 and 0.96), K ($R^2$ = 0.89 and 0.57), Ca ($R^2$ = 0.97 and 0.70), and Mg ($R^2$ = 0.80 and 0.67) in roots, while the significant and inverse relationship with $R^2$ = 0.84 and 0.70 was found between the Na concentration in roots and total biomass across both *A. modesta* and *A. vasica*, respectively. For the relationships between total biomass and P, Na, and Mn the slope was higher for *A. vasica* than for *A. modesta* while the slope of relationship between total biomass and K was higher for *A. modesta* than for *A. vasica*. However, both species had similar slopes for their relationship with Ca content and total biomass (**Figure 5**).

# DISCUSSION

Our results showed the enhanced growth of the two selected species grown in the mining waste which provides novel information about habitat restoration by utilizing the two selected woody species *A. modesta* and *A. vasica* on mining wastelands. Furthermore, our hypothesis regarding the comparative mineral uptake between the two species was partially supported. The biomass of *A. vasica* accumulated more Mg, P, and Na and less N and K than *A. modesta*, while both species accumulated similar amounts of Ca. Such species-specific mineral uptake behavior improves our understanding of the ecological restoration of mining sites with varying mineral concentrations. The enhanced growth of both species in the quarry waste, could be due to the high Ca content (2.37%) along with other nutrients such as N, P, K, and Mg and their uptake by the plants, as shown by their significant correlation with the total biomass. Furthermore, the presence of gravel in the mining soil can lower the substrate water-holding capacity, which could prevent the invasion of *Prosopis juliflora* (Khan et al., 2019).

Although mining changed the original soil strata inhabited by these species, the solid waste did not have an adverse impact on plant growth, and the treatments seemed likely to have fertilization effect. The treatment efficiency in the long terms was also observed on the growth of both species, indicating the suitability of both species for restoration of solid waste with the mentioned physiochemical properties of soil. Our findings support Richardson and Evans (1986), who utilized grasses for the restoration of limestone quarrying landscapes. They also found that the waste did not contain any toxic elements and promoted the growth of the selected species. Furthermore, our study also revealed that the solid waste areas can be converted into green patches using indigenous species without ameliorating the existing soil; some other exotic species capable of growing in soils with high Ca content could be planted to establish vegetation. These findings can help improve ecosystem restoration in limestone mining activities where habitats have been altered due to excavation.

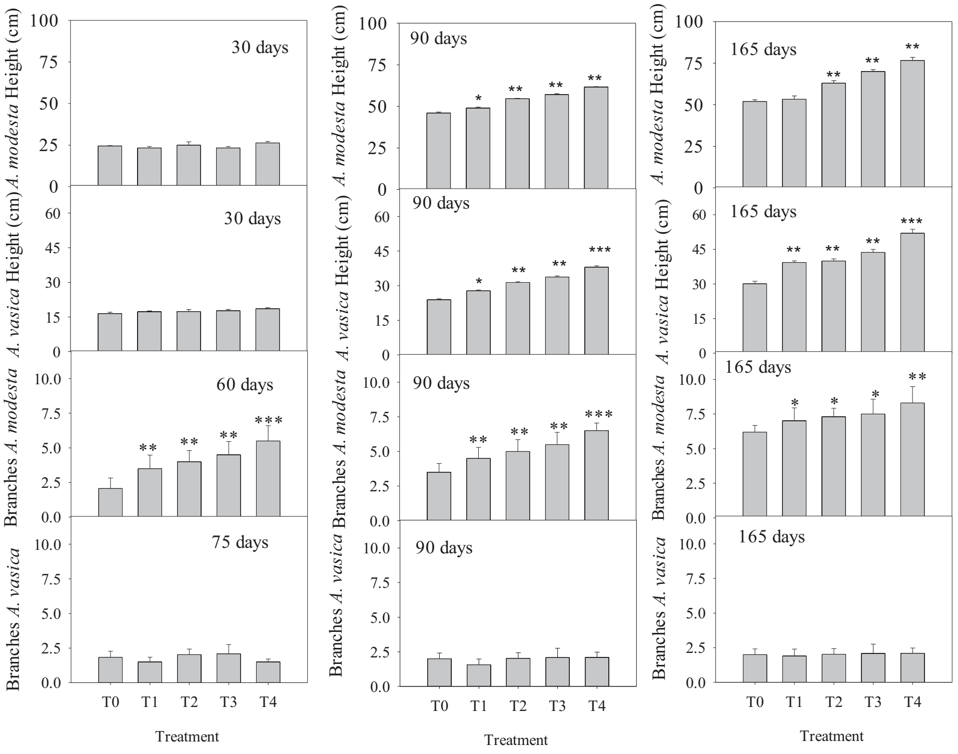

**FIGURE 3 |** Effect of quarry waste on plant height (cm) and number of branches per plant in *Acacia modesta* and *Adhatoda vasica* during the initial months, middle months, and before the harvest. The asterisk indicate the significant difference of treatment means with the control ($T_0$): *$P < 0.05$, **$P < 0.01$, and ***$P < 0.0001$.

**TABLE 3 |** The effect of solid waste treatment, duration of days, and their interactions on the growth of *Acacia modesta* and *Adhatoda vasica*.

| Species | Source | Type III SS | *df* | Mean sum of square | *F*-value | Significance |
|---|---|---|---|---|---|---|
| *Acacia modesta* | Treatment | 1173.12 | 4 | 293.29 | 51.94 | 0.000 |
| | Days | 11805.01 | 2 | 590.50 | 1045.36 | 0.000 |
| | Treatment days interaction | 564.09 | 8 | 70.51 | 12.48 | 0.000 |
| *Adhatoda vasica* | Treatment | 754.03 | 4 | 188.50 | 14.91 | 0.000 |
| | Days | 3942.76 | 2 | 1971.38 | 156.01 | 0.000 |
| | Treatment days interaction | 336.25 | 8 | 42.03 | 3.32 | 0.008 |

*For the models of both the species $R^2$ was $>0.90$.*

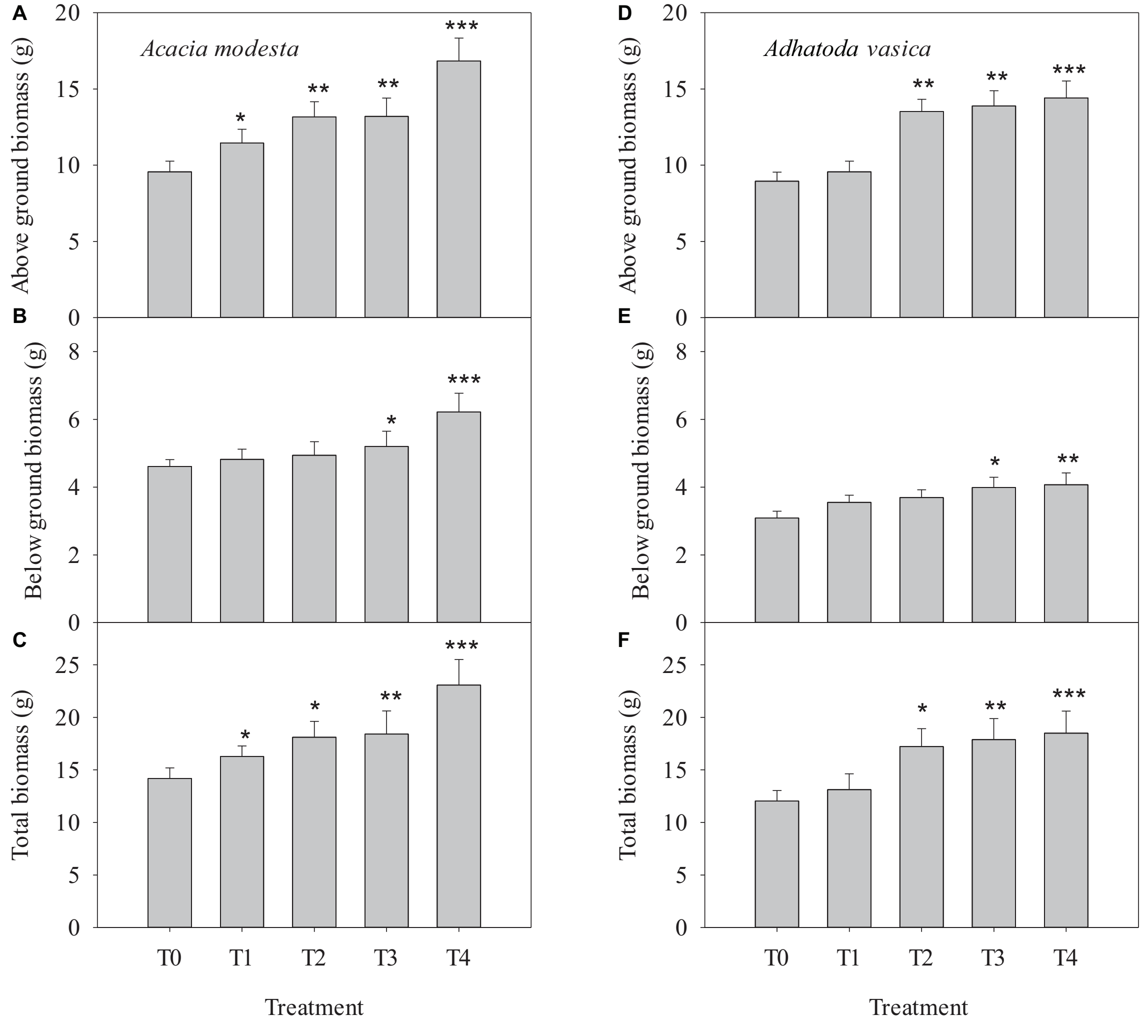

**FIGURE 4 |** Effect of quarry waste on above ground biomass, below ground biomass, and total biomass in *Acacia modesta* **(A–C)** and *Adhatoda vasica* **(D–F)**. The asterisk indicate the significant difference of treatment means with the control (T0): *$P < 0.05$, **$P < 0.01$, and ***$P < 0.0001$.

Our results are also consistent with prior ones reporting that limestone quarry waste is mainly composed of calcium carbonate, which could promote plant growth (Jim, 2001; Elsayed et al., 2017; Marcus et al., 2018). Calcium is a necessary micronutrient and is linked with various ion movements in the plasma. Its uptake can also regulate photosynthesis, carbohydrates, nitrogen assimilation, and the enzymes involved in it (Singh et al., 2018), which can enhance not only the performance of cellular mechanisms but also stress tolerance (Marschner, 1995; Konno et al., 2002; Matthew et al., 2011; Saito and Uozumi, 2020). Ecologists have classified plant species into calcifuges, which occur in soils with low Ca content, and calcicoles, which grow on calcareous soils (Lee, 1999). *A. modesta* and *A. vasica* are indigenous to the study area and have the potential to grow better in calcareous soils. However, we found that the Ca content was not uniform in both above- and belowground organs, as the roots had less Ca than shoots, which could be due to the utilization of calcium in the aerial parts for various metabolic processes (Clarkson, 1984).

Our results were consistent with those of Fu et al. (2004), in which *Pteroceltis tatarinowii* seedlings were grown in Hoagland nutrient solutions with three Ca concentrations (5, 10, and 15 mmol $L^{-1}$) or without Ca (control). Their results showed that the Ca content in the roots, leaves, and bark of *P. tatarinowii* increased with increasing Ca concentrations, and higher Ca content was found in leaves than in roots (Marschner, 1995). Kuznetsova et al. (2010) reported that indigenous *Pinus sylvestris* grew better with high Ca concentrations, and could be a candidate species for mining sites with high calcium content. The inverse correlation of total biomass with sodium concentration supports the findings of Bethke and Drew (1992) and Karim et al. (1993). As in non-halophytic species, high concentrations of sodium ions can reduce plant growth by disturbing plant water relations, unbalancing plant nutrition, and affecting several plant physiological and biochemical processes, which lead to a

**TABLE 4 |** Chemical analyses of shoot and root tissues of *Acacia modesta* grown in different concentrations of quarry waste.

| Treatment | Shoot tissues | | | | | | Root tissues | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N (%) | P (%) | K (ppm) | Na (ppm) | Ca (%) | Mg (%) | N (%) | P (%) | K (ppm) | Na (ppm) | Ca (%) | Mg (%) |
| $T_0$ | 10.90 ± 0.85e | 2.30 ± 0.01d | 432.00 ± 2.83d | 9.41 ± 0.07d | 0.56 ± 0.00e | 0.07 ± 0.00c | 14.15 ± 0.35d | 1.92 ± .01d | 426.00 ± 2.12e | 10.65 ± 0.07a | 0.36 ± 0.00e | 0.02 ± 0.00e |
| $T_1$ | 14.83 ± 0.03d | 2.55 ± 0.04c | 451.00 ± 2.12c | 9.38 ± 0.08c | 0.57 ± 0.00d | 0.07 ± 0.00bc | 18.05 ± 0.25c | 1.95 ± 0.02d | 437.00 ± 0.71d | 10.71 ± 0.01ab | 0.38 ± 0.00d | 0.035 ± 0.00d |
| $T_2$ | 18.05 ± 0.35c | 2.63 ± 0.02b | 460.00 ± 0.71b | 9.35 ± 0.06b | 0.63 ± 0.00c | 0.07 ± 0.00b | 20.81 ± 0.84b | 1.99 ± 0.01c | 447.00 ± 2.02c | 10.53 ± 0.03bc | 0.48 ± 0.00c | 0.043 ± 0.00c |
| $T_3$ | 20.86 ± 0.49b | 2.67 ± 0.07b | 469.00 ± 1.41a | 9.28 ± 0.02ab | 0.75 ± 0.00b | 0.09 ± 0.00a | 23.85 ± 0.61a | 2.06 ± 0.02b | 453.00 ± 0.71b | 10.35 ± 0.02c | 0.50 ± 0.00b | 0.06 ± 0.00b |
| $T_4$ | 22.98 ± 0.39a | 2.77 ± 0.02a | 473.00 ± 2.83a | 9.12 ± 0.01a | 0.77 ± 0.00a | 0.09 ± 0.00a | 25.14 ± 1.10a | 2.13 ± 0.02a | 461.00 ± 1.41a | 10.15 ± 0.07d | 0.70 ± 0.00a | 0.06 ± 0.00a |
| LSD | 1.90 | 0.07 | 6.59 | 0.05 | 0.01 | 0.01 | 2.11 | 0.04 | 1.96 | 0.17 | 0.01 | 0.004 |

*Different letters in the same column are significantly different at P = 0.05; LSD, least significant difference.*

**TABLE 5 |** Chemical analyses of shoot and root tissues of *Adhatoda vasica* grown in different concentrations of quarry waste.

| Treatment | Shoot tissues | | | | | | Root tissues | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N (%) | P (%) | K (ppm) | Na (ppm) | Ca (%) | Mg (%) | N (%) | P (%) | K (ppm) | Na (ppm) | Ca (%) | Mg (%) |
| $T_0$ | – | 2.71 ± 0.02e | 371.00 ± 0.71 | 13.30 ± 0.06a | 0.43 ± 0.00e | 0.10 ± 0.00e | 8.00 ± 0.28c | 2.18 ± 0.03e | 361.0 ± 2.82d | 13.31 ± 0.28a | 0.28 ± 0.00e | 0.06 ± .00d |
| $T_1$ | 8.50 ± 0.07cd | 2.89 ± 0.04d | 375.50 ± 0.70 | 13.05 ± 0.05a | 0.49 ± 0.00d | 0.12 ± 0.00d | 9.70 ± 0.84b | 2.34 ± 0.04d | 367.0 ± 1.41c | 13.15 ± 0.07a | 0.30 ± 0.00d | 0.06 ± 0.00cd |
| $T_2$ | 9.45 ± 0.08c | 2.98 ± 0.01c | 380.50 ± 0.80 | 12.85 ± 0.06a | 0.51 ± 0.00c | 0.13 ± 0.00c | 11.20 ± 0.42b | 2.60 ± 0.02c | 370.0 ± 1.70c | 12.90 ± 0.14ab | 0.34 ± 0.00c | 0.06 ± 0.00bc |
| $T_3$ | 12.91 ± 0.11b | 3.12 ± 0.04b | 389.00 ± 1.41 | 12.15 ± 0.29b | 0.58 ± 0.00b | 0.14 ± 0.00b | 13.45 ± 0.49a | 2.73 ± 0.02b | 377.0 ± 1.41b | 12.50 ± 0.14b | 0.47 ± 0.00b | 0.07 ± 0.00ab |
| $T_4$ | 14.80 ± 0.99a | 3.31 ± 0.02a | 408.00 ± 2.83 | 10.95 ± 0.30c | 0.61 ± 0.00a | 0.15 ± 0.00a | 14.0 ± 0.57a | 2.85 ± 0.01a | 404.0 ± 2.82a | 11.75 ± 0.35c | 0.57 ± 0.00a | 0.07 ± 0.00a |
| LSD | 1.33 | 0.03 | 4.16 | 0.47 | 0.007 | 0.005 | 1.63 | 0.09 | 5.48 | 0.54 | 0.002 | 0.004 |

*Different letters in the same column indicate significant differences among the means at P = 0.05; LSD, least significant difference.*

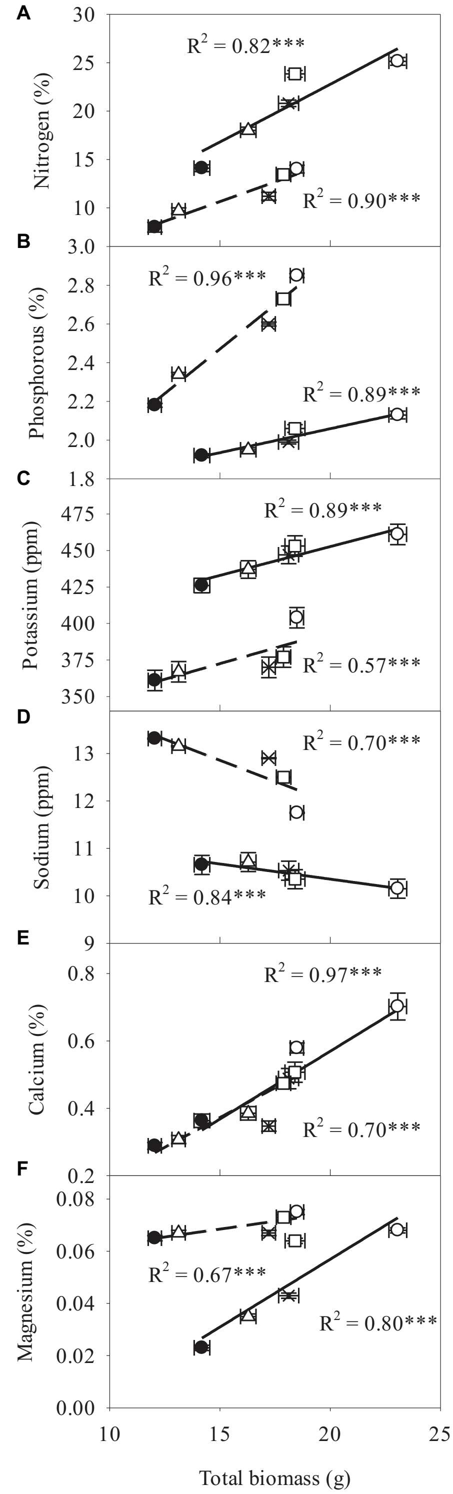

**FIGURE 5 |** Relationship of total plant oven-dried biomass with nitrogen **(A)**, phosphorus **(B)**, potassium **(C)**, sodium **(D)**, calcium **(E)**, and magnesium **(F)** in the roots of *Acacia modesta* (solid lines) and *Adhatoda vasica* (dashed lines) in $T_0$ (filled circles), $T_1$ (empty triangles), T2 (multiplication symbol), T3 (empty squares), and T4 (empty circles). Each symbol indicates the mean ± SE. $^{***}P < 0.0001$, all the relationships were significant. Linear function was fitted for all the relationships.

reduction in plant growth (Misra et al., 2006; Taffouo et al., 2009). Furthermore, the garden soil had more sodium than the solid waste, hence, the decrease in sodium content with the decrease in garden soil in the three treatments, resulted in increased biomass.

Compared with *A. modesta*, *A. vasica* absorbed higher amounts of all minerals from the mining waste, as shown in **Figures 5A–F**. Kasowska et al. (2018) reported some species that could uptake comparatively more minerals from mining waste. *A. vesica* being the shrubby species and considered to be the initial colonizer could have the potential for higher uptake of minerals, except calcium, as both species had similar uptake of Ca. Although an inverse relationship was found between the biomass and the Na uptake, *A. vasica* had a larger uptake of Na than *A. modesta* in a given treatment, indicating its potential to uptake the growth-reducing minerals more than the later colonizers such as trees. The results of the present study show that both *A. modesta* and *A. vasica* will be helpful for establishing vegetation on limestone quarry sites with high calcium content.

## CONCLUSION

Both native species (*A. modesta* and *A. vasica*) showed increased growth in limestone quarry waste compared with the control. Adaptive to the mining substrate, both of these species can be utilized for the restoration of such mined landscapes without any soil amelioration. Planting these species will not only provide an ecological solution for the disposal of the limestone quarry waste but will also create opportunities for other environmental goods and services, such as timber production, habitat formation, carbon sequestration, erosion control, and natural ecosystem functioning.

## DATA AVAILABILITY STATEMENT

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

## AUTHOR CONTRIBUTIONS

MH, ZS, and AK designed the experiment. MH and ZS did the field work. MH and RM conducted the laboratory analysis. MH, ZS, AK, and K-FC analyzed and interpreted the data. All authors contributed the manuscript writing.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.637833/full#supplementary-material

**Supplementary Figure 1 |** Effect of quarry waste on plant height (cm) of *Acacia modesta* over time. The asterisks indicate the significant difference of treatment means with the control ($T_0$): *$P < 0.05$, **$P < 0.01$, and ***$P < 0.0001$.

**Supplementary Figure 2 |** Effect of quarry waste on number of branches per plant in *Acacia modesta* over time. The asterisks indicate the significant difference of treatments means with the control ($T_0$): *$P < 0.05$, **$P < 0.01$, and ***$P < 0.0001$.

**Supplementary Figure 3 |** Effect of quarry waste on plant height of *Adhatoda vasica* over time. The asterisks indicate the significant difference of treatments means with the control ($T_0$): *$P < 0.05$, **$P < 0.01$, and ***$P < 0.0001$.

**Supplementary Figure 4 |** Effect of quarry waste on the number of branches per plant in *Adhatoda vasica* over time.

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.