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Research Article

Growth and survivorship of *Vetiveria zizanioides* in degraded soil by gold-mining in the Peruvian Amazon

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Abstract

In the Peruvian Amazon, large area of primary forest have been deforested by Artisanal and small-scale gold mining (ASGM). *Vetiveria zizanioides* (Poaceae) is considered an excellent plant for the ecological restoration of degraded lands. The present study aimed to analyze the growth and survivorship of *V. zizanioides* in degraded soils by gold-mining in the Peruvian Amazon (Madre de Dios). The experiment was conducted under greenhouse conditions, and it followed a randomized complete block design with four treatments (substrates). The substrates were sand (mining), sand+pebbles (mining), forest soil, and amended soil. We evaluated the survivorship, shoot, tiller, and biomass production of *V. zizanioides* for two months. Univariate analysis of variance was used to detect differences among treatments. Fifteen days after experiment establishment, *V. zizanioides* survivorship was significantly higher in ASGM substrate 2 (sand+pebbles) than in other substrates, following the order of ASGM substrate 1 (sand) > forest soil > amended soil. However, at the end of the experiment, we did not find a significant difference on survivorship in subsequent assessments, and the total biomass per plant was lower in mining substrates than in non-mining substrates. The total biomass was significantly higher in amended soil than in other substrates, with yields between 1.7 and 3.6 times more biomass in amended substrate than in mine substrates. The soil substrate type significantly affected the survivorship, number of shoots, and biomass of *V. zizanioides*. The research results can provide a reference for remediation of degraded soils by gold-mining in the Peruvian Amazon.

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Introduction

For many decades, the mining industry has played an important role in the world economy and the development of human societies (Cherlet et al., 2018; Iatan, 2021). Artisanal and small-scale gold mining (ASGM) is widespread around the world (Cherlet et al., 2018), mainly in developing countries where it is an essential part of the local economy. Peru is recognized as the largest gold producer in South

America and seventh-largest globally (Cortes-McPherson, 2020), but most of Peruvian's gold production is extracted by ASGM (Csillik and Asner, 2020).

The Peruvian Amazon rainforest is considered one of the most biodiverse places in the world (Riley-Powell et al., 2018). Nevertheless, in the last 15 years, ASGM has grown explosively, driven by rising gold prices and increased road connectivity (Álvarez-Berríos et al., 2021). In the Peruvian Amazon, a large area of primary forest has been deforested by ASGM (Caballero et al., 2018) because the mine activities remove soil and aboveground vegetation (Garate-Quispe et al., 2021), especially in Madre de Dios. Madre de Dios is responsible for 70% of Peruvian's gold production (Swenson et al., 2011).

Species of Poaceae (grasses) are considered as potential phytoremediators of heavy metals and ecological restoration in contaminated mining lands (Patra et al., 2021; Sharma et al., 2021). Grasses can be grown in degraded soils where trees and cannot be established (Issaka and Ashraf, 2021); they have the capability of rapid coverage of the contaminated surface, fast growth, production of high biomass (Amaral et al., 2019; Hao et al., 2020; Patra et al., 2021) and improve soil physical properties during early restoration state (Hao et al., 2020).

On the other hand, *Vetiveria zizanioides* (L.) Nash is considered an excellent plant for the ecological restoration of degraded lands (Issaka and Ashraf, 2021; Patra et al., 2021). Previous studies demonstrated that *V. zizanioides* tolerate soils highly contaminated by mining activities in South America (Brandt et al., 2006; Amaral et al., 2019; Milla-Moreno and Guy,2021) and other continents (Hao et al., 2020; Wu et al., 2021). In this experimental study, *V. zizanioides* was selected due to its capability to endure harsh environments, remove heavy metals, control erosion, and allow other species to get established (Brandt et al., 2006; Peña-Salamanca et al., 2013; Almeida et al., 2019; Patra et al., 2021).

At present, although there are many studies on utilizing trees, shrubs, and crops plants for reforestation of abandoned mining lands (Garate-Quispe et al., 2021; Román-Dañobeytia et al., 2021; Velásquez-Ramírez et al., 2021), there are focused on the recovery of the forest cover, and there is no information about phytoremediation of heavy metals. Therefore, prior to use *V. zizanioides* in reforestation and phytoremediation projects, experimental studies are needed to evaluate the survival and growth of this species in soils highly degraded by gold mining.

The present study aimed to analyze the growth and survivorship of *Vetiveria zizanioides* (L.) Nash in degraded soil by gold-mining in the Peruvian Amazon. We hypothesized that (1) survival and growth were affected by the soil treatments, and (2) biomass production was higher in reference soil than in mining soils.

Materials and Methods

Study site

The experiment was conducted from June 2011 to August 2011 for 60 days under greenhouse conditions at a forest seedling nursery belonging to the Peruvian Amazon Research Institute (IIAP) in the region of Madre de Dios, southwestern Peruvian Amazon (12º39'5" S 69º19'16" W and an altitude of 230 m above sea level).

Soil substrates

In the present study, four substrates were used, (1) sand, (2) sand + pebbles, (3) forest soil, and (4) amended soil. The substrates sand and sand + pebbles were obtained from degraded gold-mining areas located in the Huepetuhe district, 135 km away from the nursery (IIAP). The sand (mining) substrate was composed of only sand from mined areas. The sand + pebbles (mining) substrate was composed of 30% sand and 70% pebbles from mined areas. The forest soil was obtained from forests near the nursery (IIAP). Amended soil was obtained from a mixture of decomposed organic matter (50%), ashes (10%), sawdust (10%), and forest soil (30%).

Species

Vetiveria zizanioides (L.) Nash propagation material was obtained from a seedling nursery at the scientific station of the IIAP. The *V. zizanioides* plants used in the present study developed from rooted shoots obtained by splitting twelve mother plants (Figure 1). The 800 plants obtained from the mother plants were pruned prior to planting; plants had a 10 cm of the shoot and 10 cm long root system. After pruning, they were immersed in water for 24 hours, and subsequently, vetiver shoots were planted in polybag containers (0.8 L) containing the prepared substrates (Figure 1).

Experimental design

The experiment followed a randomized complete block design with four treatments (4 types of substrates), where two types of substrates were from degraded gold-mining areas. The other substrates were from forest soil and amended soil. Each treatment was represented by ten individuals, replicated in 20 blocks, for a total of 800 individuals, and treatments locations were fully randomized. The plants were drip-irrigated every 15 days to simulate the low humidity soil that exists in abandoned mining areas.

Data collection

We evaluated the survivorship, shoot, tiller, and biomass production of *V. zizanioides* plants for two months. Assessment of survivorship, shoot, and tiller production was made every 15 days. While the biomass production assessment was made at the end of the experiment.

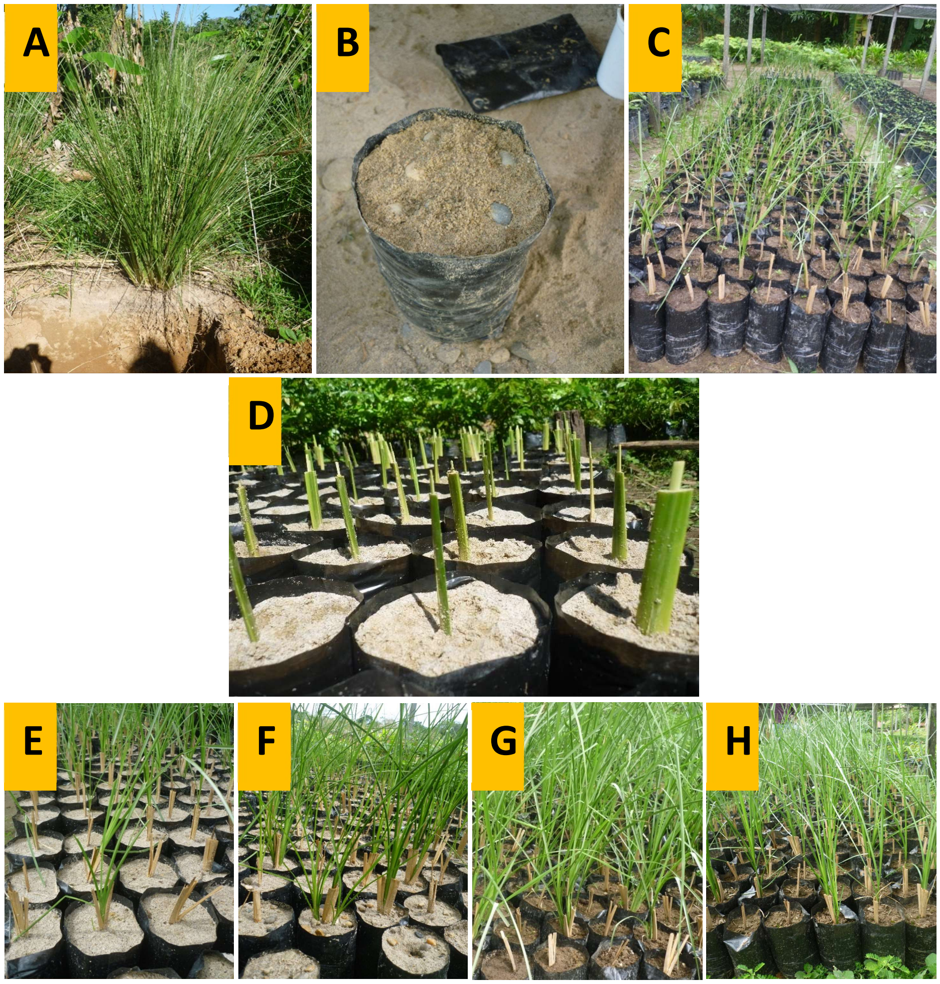

Figure 1. Images about the methodology followed for the present study. (A) Mother plants of *Vetiveria zizanioides.* (B) Filling of polybag containers with substrates. (C y D) Arrangement of the polybag containers according to the experimental design. *Vetiveria zizanioides* growth after two months according to treatment: (E) ASGM sand substrate, (F) ASGM sand+pebbles substrate, (G) forest soil, and (H) amended soil.

For biomass analysis, 30 *V. zizanioides* plants were randomly selected from each treatment. At harvest, the plants were carefully removed from the polybag containers, washed with water to remove any attached particles, and freeze-dried to a constant weight at 65ºC.

Statistical analysis

Univariate analysis of variance (ANOVA) followed by Student-Newman-Keuls (SNK) pairwise comparison method ($P < 0.05$) was used to detect differences in survivorship and shoots and tillers production. The effect of substrate treatments on biomass accumulation was examined using the non-parametric Kruskal-Wallis test, followed by the Student-Newman-Keuls pairwise comparison method. All variables were tested for normality and equal variance between treatments, using the Shapiro test and Bartlett's test, respectively. Statistical analyses were performed in the R. 4.0 software package (R Core Team, 2020), figures were made using the *ggplot2*.

Results

The soil substrate type had a significant effect on the survivorship (Figure 2), the number of shoots (Figure 3a), and biomass (Figure 4) of *V. zizanioides*. However, it had no significant effects on the number of tillers (Figure 3B).

Survival

Fifteen days after experiment establishment, *V. zizanioides* survivorship varied significantly by the effect of treatments (ANOVA, $P < 0.05$). On average, *V. zizanioides* survivorship was significantly higher in ASGM substrate 2 (sand+pebbles) than in other substrates, following the order of ASGM substrate 1 (sand) > forest soil > amended soil (Figure 2). However, we did not find a significant difference in survivorship of *V. zizanioides* in subsequent assessments (ANOVA, $P < 0.05$) (Figure 2).

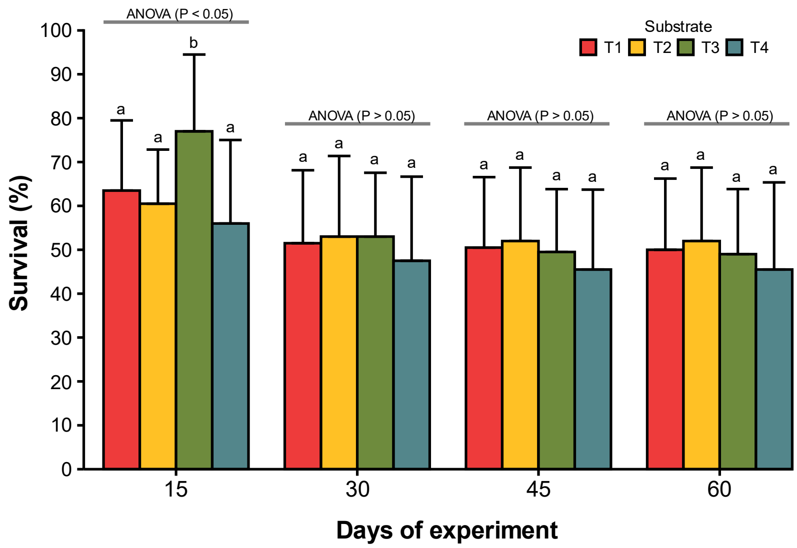

Figure 2. Survivorship of *Vetiveria zizanioides* as a function of time under four different substrates from degraded gold-mining, forest soil and amended soil in the Peruvian Amazon. Values are means ± standard deviation. T1: ASGM sand substrate 1; T2: ASGM sand+pebbles substrate; T3: forest soil; T4: amended soil. Different letter in the same group indicates a significant difference at 5% level according to Student-Newman-Keuls (SNK) *post hoc* test.

Shoots and tillers

The type of substrate significantly influenced shoots production (ANOVA, P < 0.05). Interestingly, the highest yielding *V. zizanioides* was in mining substrates, followed without significant difference by forest soil substrate (Figure 3A). However, the number of shoots was significantly higher in the mining substrates than in amended soil (Figure 3A).

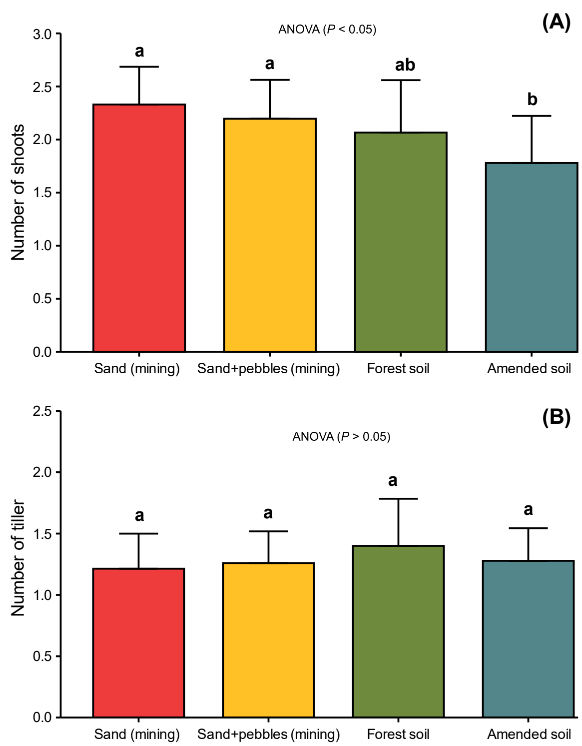

Figure 3. Effects of different substrates (mining and non-mining) on shoots (A) and tiller (B) production of *V. zizanioides* in 60 days. Values are means ± standard deviation. Different letter in the same group indicates a significant difference at 5% level according to ANOVA test, and Student-Newman-Keuls (SNK) posthoc test.

Biomass

The results in the biomass reported as dry weight per plant of *V. zizanioides* are presented in Figure 4. At the end of the experiment, the total biomass per plant differed significantly among treatments ($P < 0.05$, Figure 4), and it was lower in mining substrates (sand and sand+pebbles) than in non-mining substrates. The total biomass was significantly higher in amended soil than in other substrates ($P < 0.05$), with yields between 1.7 and 3.6 times more biomass in amended substrate than in mine substrates. On the other hand, the total dry biomass of *V. zizanioides* did not differ significantly ($P > 0.05$) between forest soil and sand+pebbels mining substrate. However, the use of sand mining substrate resulted in lower biomass production in *V. zizanioides* than in the other three substrates ($P > 0.05$, Figure 4).

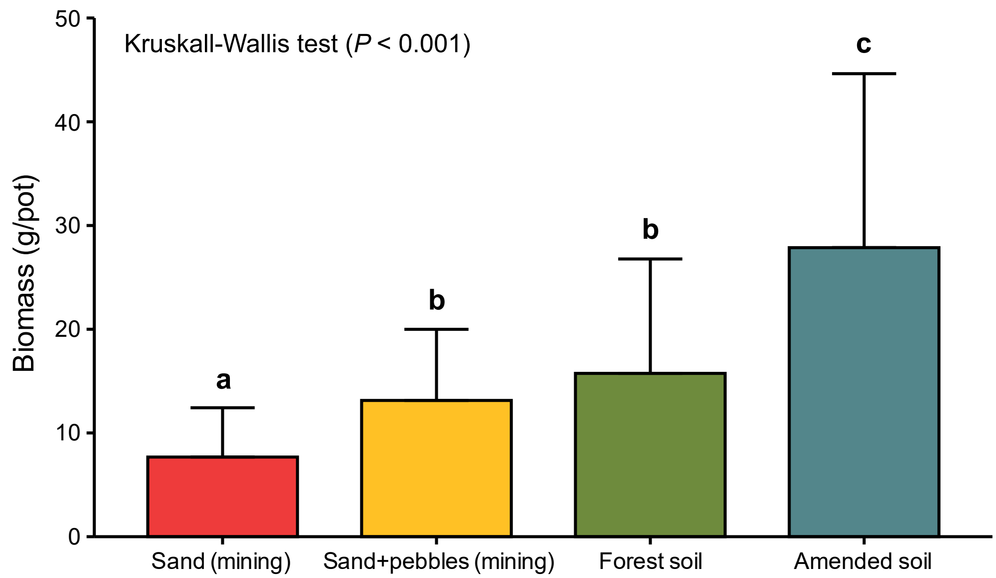

Figure 4. Effects of different substrates (mining and non-mining) on total biomass accumulation of *V. zizanioides* in 60 days. Values are means ± standard deviation. Different letter in the same group indicates a significant difference at 5% level according to Kruskall-Wallis test, and Student-Newman-Keuls (SNK) *post hoc* test.

Discussion

For many decades, the mining sector has been a significant part of the economy (Iatan, 2021). However, in the Peruvian Amazon large area of soil has been degraded and contaminated with heavy metals because of the mine activities, especially in Madre de Dios (Alarcon et al., 2021; Velásquez-Ramírez et al., 2020). Previous studies have been demonstrated that *V. zizanioides* can withstand harsh environments such as gold-mining sites (Chiu et al., 2006) due to its high tolerance to a wide range of edaphic and climatic conditions (Brandt et al., 2006; Torres et al., 2010). These characteristics make *V. zizanioides* a promising species for phytoremediation of degraded lands by gold-mining in Madre de Dios.

Survival

After 60 days, the type of substrate did not significantly influence the survival of *V. zizanioides* (ranged from 46% to 52%) (Figure 2). At mining substrates mean *V. zizanioides* survival percentage ranged from 50% to 52%. This result provided an important evidence that *V. zizanioides* can survive in soils from degraded areas by gold-mining, with lower acidity (higher pH), lower cation exchange capacity, and lower clay and silt, and lower soil organic matter content than the reference forest (Román-Dañobeytia et al., 2021). The ability of *V. zizanioides* to tolerate contaminated soils has been demonstrated by Datta et al. (2011) in a greenhouse study about the phytoremediation potential in arsenic-contaminated soils. It suggests that *V. zizanioides* can be used on gold-mining degraded lands with minimal or without fertilizing (Truong and Thai-Danh, 2015; Xia, 2004) because the mean survival percentage was similar among the substrates (ASGM substrates, forest soil, and amended soil). However, the survival rates found in the present study are lower than those reported in the previous study in highly contaminated sites. Troung and Thai-Danh (2015) reported that after three months of *V. zizanioides* planting on highly contaminated copper waste rocks (Chile), about 80% of cultivated Vetiver plants survived and grew well. In a study of recovery of a degraded ecosystem of an oil shale mined land in Southwest of China, Xia (2004) found a high survival rate of *V. zizanioides* (> 90%).

Shoots and tillers

After 60 days, the type of substrate significantly influenced shoot production, but in the number of tillers, we did not find differences among treatments. The higher shoots production of *V. zizanioides* in mining substrates than amended soil substrate suggests that *V. zizanioides* has an outstanding ability to survive in various types of soils (Srivastava et al., 2008). *V. zizanioides* grows well in sandy loam soil (Pandey and Praveen, 2020). Therefore, the sand mining substrate would promote shoots to develop rapidly (Truong and Thai-Danh, 2015). Similarly, Dudai et al. (2006), in an experimental study under Mediterranean conditions, found that *V. zizanioides* produce a higher number of shoots in sandy than in loamy sand soils.

Biomass

Biomass production of *V. zizanioides* was lower in the mining substrates. The amended soil was the most favorable substrate for biomass yield, where yields exceeded those of mining soils by an average of 115% in the sand substrate and 250% in sand+pebbles substrate. It suggests that this species has a high potential to adapt to the degraded environment by gold-mining, due to its high capacity for vegetative reproduction (Brandt et al., 2006). The lower biomass production of *V. zizanioides* in mining soils confirms that fertilization is crucial in order to promote plant growth of this species on low fertility substrates (Srivastava et al., 2008; Amaral et al., 2019). Experimental studies found similar results in lead-contaminated soils in Thailand (Rotkittikhun et al., 2007) and oil shale mined land in China (Xia, 2004), who observed a significant increase in biomass between 25% to 27% in pig manure treatments and Fertilizer application. On the other hand, probably due to more extreme soil conditions in sand subtrate, particularly physical conditions, lower soil organic matter and cation exchange capacity (Román-Dañobeytia et al., 2021; Velásquez-Ramírez et al., 2020), we found a higher biomass production of *V. zizanioides* in sand+pebbels than in sand substrate. In Madre de Dios, previous studies in gold-mining degraded lands found that: (1) the soil organic matter is higher in sand+pebbles (ranged from 1.3 to 1.9 %) than in sandy soil (ranged from 0.2 to 0.48 %); (2) the cation exchange capacity is higher in sand+pebbles (ranged from 10.7 to 12.4 Cmol/kg) than in sandy soil (ranged from 4.3 to 6.4 Cmol/kg) (Román-Dañobeytia et al., 2021; Velásquez-Ramírez et al., 2020).

Conclusion

The soil substrate type significantly affected the survivorship, number of shoots, and biomass of *V. zizanioides*. The research results can provide a reference for remediation of degraded soils by gold-mining in the Peruvian Amazon. According to the results presented herein, it can be concluded that vetiver is an excellent plant species for the reclamation of gold-mining lands in Madre de Dios. Especially in soils with sand+pebbles because we found that similar production to the forest soil. Therefore, *V. zizanioides* can be used for rehabilitation of abandoned gold-mining areas in Madre de Dios, which are often high in heavy metals and low in plant nutrients.

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