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Plants from the abandoned Nacozari mine tailings: evaluation of their phytostabilization potential

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Phytostabilization is a remediation technology that uses plants for in-situ stabilization of contamination in soils and mine tailings. The objective of this study was to identify native plant species with potential for phytostabilization of the abandoned mine tailings in Nacozari, Sonora in northern Mexico. A flora of 42 species in 16 families of angiosperms was recorded on the tailings site and the abundance of the most common perennial species was estimated. Four of the five abundant perennial species showed evidence of regeneration: the ability to reproduce and establish new seedlings. A comparison of selected physicochemical properties of the tailings in vegetated patches with adjacent barren areas suggests that pH, electrical conductivity, texture, and concentration of potentially toxic elements do not limit plant distribution. For the most abundant species, the accumulation factor for most metals was < 1, with the exception of Zn in two species. A short-term experiment on adaptation revealed limited evidence for the formation of local ecotypes in Prosopis velutina and Amaranthus watsonii. Overall, the results of this study indicate that five native plant species might have potential for phytostabilization of the Nacozari tailings and that seed could be collected locally to revegetate the site. More broadly, this study provides a methodology that can be used to identify native plants and evaluate their phytostabilization potential for any mine tailings site.

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ABSTRACT

Phytostabilization is a remediation technology that uses plants for *in-situ* stabilization of contamination in soils and mine tailings. The objective of this study was to identify native plant species with potential for phytostabilization of the abandoned mine tailings in Nacozari, Sonora in northern Mexico. A flora of 42 species in 16 families of angiosperms was recorded on the tailings site and the abundance of the most common perennial species was estimated. Four of the five abundant perennial species showed evidence of regeneration: the ability to reproduce and establish new seedlings. A comparison of selected physicochemical properties of the tailings in vegetated patches with adjacent barren areas suggests that pH, electrical conductivity, texture, and concentration of potentially toxic elements do not limit plant distribution. For the most abundant species, the accumulation factor for most metals was < 1, with the exception of Zn in two species. A short-term experiment on adaptation revealed limited evidence for the formation of local ecotypes in *Prosopis velutina* and *Amaranthus watsonii*. Overall, the results of this study indicate that five native plant species might have potential for phytostabilization of the Nacozari tailings and that seed could be collected locally to revegetate the site. More broadly, this study provides a methodology that can be used to identify native plants and evaluate their phytostabilization potential for any mine tailings site.

Key words: Copper mine tailings, phytostabilization, Nacozari, Sonora.

INTRODUCTION

aeolian

Unreclaimed mine tailings represent an important environmental problem as eolian dispersion and water erosion may transfer potentially toxic elements into local trophic webs and nearby human settlements. Reference? Phytostabilization is a form of remediation that involves the use of plants for *in-situ* stabilization of tailings and contaminants (Mendez & Maier, 2008). Implementing phytostabilization in a particular tailing requires identification of suitable plant species for specific ecological conditions as well as the appropriate amendments to allow plant germination and growth. In arid and semiarid environments, plants suitable for phytostabilization should be native, drought-, salt- and metal tolerant and should limit shoot metal accumulation (Mendez & Maier, 2008).

metal-tolerant

I would question the importance of nativity. They certainly shouldn't be invasive but in highly modified "novel ecosystems" (sensu Hobbs et al. 2009; see http://dx.doi.org/10.1016/j.tree.2009.05.012) might non-native species be considered if they provide appropriate ecosystem function and desirable levels of phytostabilisation?

Surveys of native plants that naturally colonize mine tailings offer the opportunity to identify species with potential in phytostabilization (Carrillo-Gonzalez & Gonzalez-Chavez, 2006; Cortes-Jimenez et al., 2013). Using native species has several advantages including adaptation to local environmental conditions and avoiding the introduction of invasive species that may affect local plant communities. Analysis of the pattern of metal accumulation among plants growing in mine tailings allows identification of species with the best potential for phytostabilization (Santos-Jallath et al., 2012; Cortes-Jimenez et al., 2013). If

You should define these concepts

bioconcentration and translocation factors are greater than 1, species are not suitable for phytostabilization but may have potential for phytoextraction; in contrast, if these ratios are less than one and if metal concentrations in plant tissues do not reach animal toxicity levels, species have potential for phytostabilization (Mendez & Maier, 2008).

Studies on the relationships between plant abundance and the physicochemical properties of mine waste have identified some of the factors that limit plant establishment in mine tailings (Conesa, Faz & Arnaldos, 2006; Anawar et al., 2013; Parraga-Aguado et al., 2013). Some of the tailing properties that have been found to influence plant abundance and distribution include pH (Conesa, Faz & Arnaldos, 2006), salinity (Parraga-Aguado et al., 2013) and metal concentration (Ortiz-Calderon, Alcaide & Li-Kao, 2008). Identifying the physicochemical properties of tailings that restrict plant establishment may be critical for the implementation of phytostabilization and for understanding the type of amendments needed to facilitate seedling establishment (Parraga-Aguado et al., 2013; Gil-Loaiza et al., 2016).

It would be valuable if you could briefly describe what such amendments might consist of

Studies of plant adaptation to local mine wastes have important implications for the implementation of phytostabilization. The mechanism involved in the evolution of metal tolerance has been documented in many plant species (Antonovics, Bradshaw & Turner, 1971; Baker, 1987). Metal tolerant populations evolve through natural selection in response to high levels of metals in mine wastes (Ke et al., 2007). If metal tolerant ecotypes have evolved locally, phytostabilization should take advantage of such local ecotypes as they provide better cover and persistence than commercial varieties when grown in mine wastes (Smith & Bradshaw, 1979). Thus, the implementation of phytostabilization should take into account whether local plant ecotypes have evolved in particular mine tailings.

Past and current mining activities in northern Mexico have generated large amounts of unconfined mine wastes that poses risks to human populations and adjacent ecosystems (Jimenez et al., 2006). The Nacozari region in northeastern Sonora, hosts important copper deposits and one of the most important copper mines in northwestern Mexico. The

Moctezuma Copper Company operated the Pilares mine east of Nacozari from 1900 to 1949 and generated several million tons of waste distributed in three tailings deposits that cover 52 ha around the town (Alvarado & Volke, 2004; De la O Villanueva et al., 2013). Selected physicochemical parameters from these tailings have been described, including mineral composition (Romero et al., 2008), texture (De la O Villanueva et al., 2013), pH, electrical conductivity and metal content (Meza-Figueroa et al., 2009). Although metal concentration in tailings is relatively low, the seasonal formation of efflorescent salts represents a serious problem; these salts can have high metal concentrations and are subject to wind dispersion which can move toxic metals from the tailings into nearby residential soils (Meza-Figueroa et al., 2009). Meza-Figueroa et al. (2009) suggest that one alternative for prevention of off-site eolian dispersion and water erosion of the tailings is to create a vegetative cap using native plants. However, knowledge of the native plants growing near or on the Nacozari tailings and their potential in phytostabilization is limited.

Native plants have colonized the Nacozari tailings, although the distribution is localized and patchy and is not sufficient to prevent eolian dispersion or water erosion. The overall goal of this study is to identify plant species with potential for phytostabilization of this site. Our objectives are to: a) describe the taxonomic composition of the plant species growing in the Nacozari tailings; b) describe the abundance and population structure of the most common species; c) explore the physicochemical parameters that may limit plant distribution; d) describe the pattern of metal accumulation in the most common plant species and e) evaluate through short-term experiments whether local ecotypes have evolved in two of the plant species.

MATERIAL AND METHODS

Study area. -The Nacozari mining district is located in northeastern Sonora, 123 km south of the Arizona (USA) border. The regional climate is semi-arid (BS1) with the mean temperature in Nacozari ranging from 12.1ºC during January to 27.9ºC during June. Mean annual rainfall is 578 mm with more than 60% occurring during July, August and September (Servicio Meteorologico Nacional, 2015). Regional vegetation is foothills thornscrub at lower elevations and oak woodland at higher elevations (Martinez-Yrizar, Felger & Burquez, 2010).

It would be helpful if you described the dominant species associated with these plant communities

The mining district hosts important ore deposits, including porphyry copper, breccia pipe and veins with Cu, Mo, Au, Ag and Zn (Alvarado & Volke, 2004). The Pilares copper ore deposit (0.7-1.2 % Cu) was discovered in 1886 and purchased years later by the Moctezuma Copper Company (Phelps Dodge subsidiary). Mining activity lasted around 50 years, producing around 3000 ton/day of copper until the mine was closed in 1949. Large amounts of waste were distributed into three deposits around the town of Nacozari and then abandoned (Alvarado & Volke, 2004).

The three tailing deposits differ in size: 1) the center tailings deposit is medium-sized and located within the urban area, at the southern margin of town, 2) the southern deposit is the smallest and is located just south of Nacozari along the Moctezuma-Agua Prieta road, and 3) the southeastern deposit is the largest and is located along the Nacozari-La Caridad road (Alvarado & Volke, 2004). Although the three deposits have been studied (Alvarado & Volke, 2004), work has concentrated on the center tailings deposit because of its proximity to the town (Meza-Figueroa et al., 2009). The mineral composition of this deposit is mainly quartz (SiO_2), gypsum ($CaSO_4{\cdot}2H_2O$), lepidocrocite ($\Upsilon FeO[OH]$) and copper sulfate ($CuSO_4$, Romero et al., 2008). Mean pH is 3.8 ± 0.3 and mean electrical conductivity is 340.1 ± 2 µS/cm (Meza-Figueroa et al., 2009). Texture analysis revealed that 80% of the material is coarse grained with significant variation in particle size in the center tailing from coarse sand to fine silt (De la O-Villanueva et al., 2013). The most common metals in the center deposit are Fe (31,739 ± 381.9 mg/kg), Cu (400.5 ± 15.8 mg/kg), Rb (298.4 ± 5.6) and Mn (158.5 ± 10.5 mg/kg); mean values of As and Pb are 29.3 ± 4 and 39 ± 4.2 mg/kg respectively (Meza-Figueroa et al., 2009). However, some metals including Cu, Mn, Zn and Ba, reach very high values in efflorescent salts (e.g., Cu: 68,751 ± 865 mg/kg), and are highly susceptible to eolian transport into nearby residential soils (Meza-Figueroa et al., 2009).

central?

This study focused on the center deposit which has an area of approximately 19 ha, a volume of 1.5 million m^3 and a mass of 3.3 million tons (De la O-Villanueva et al., 2013). This tailing is located at 30°22'28" N and 109°41'15" W at an elevation of 1050 masl. The entire deposit was explored for perennial plants resulting in the identification of four patches that contained all plants. Patch size varied from 34 to 743 m^2 for a total of 1591 m^2 or 0.84% of the total area of the deposit.

This is a little unclear - are you saying that all the species found across the whole site were represented in four patches or that there were just four patches of vegetation?

Plant inventory. – The tailings were visited six times during the year in order to collect specimens of perennial species from each of the four patches during the flowering season. Annual species were collected during the summer rainy season. Species were identified using local floras (*i.e.* for trees: Felger, Johnson & Wilson, 2001) or by comparison with specimens deposited at the University of Sonora Herbarium.

Abundance and population structure. – The abundance of perennial species was determined using 10 x 10 m sampling plots throughout all four patches. As many sampling plots were used as was necessary to obtain a census of all perennial plants in the entire patch. Within each plot the identity of each individual was recorded and its height was measured with a metric tape for shrubs and a graduated telescoping pole for trees. For annual species, a 1 m^2 sampling plot was used randomly distributed within each patch. We used a total of 6 (1 m^2) plots per patch. For this set of plants only the identity of each individual and the number of plants per plot were recorded.

Please describe how the locations for the 10 x 10 m plots were selected. It sounds like there were differences in sampling intensity between patches. That and the differences in patch size will affect the number of plant species present in each plant (cf. species-area relationship). What implications, if any, might this have for your analysis?

Relation between plant distribution and physicochemical properties of tailings. – We compared several physicochemical properties of tailings in patches with vegetation and in areas without vegetation. We used a paired sampling approach taking tailings samples (0-20 cm in depth) from each patch with perennial plants and adjacent areas with no vegetation. For each pair we took 3 randomly located samples that were combined to form a composite sample (1 kg); in total we obtained four composite samples from plant patches and four composite samples from areas without vegetation (n=8). Once in the lab, samples were dried and homogeneized. The pH and electrical conductivity (EC) were determined in solid suspensions (1:20 solid:water) using a 100 Ecosense pH meter and a portable conductivity meter (Hanna), respectively. Particle size distribution was determined through wet sieving following the method of Beare & Bruce (1993). For metal analysis, homogenized tailing samples were placed in plastic bags and measured directly with a field portable X-ray fluorescence (PXRF) analyzer NITON XL3t. The procedure followed the manufacturer instructions and the recommendations of the EPA-6200 method (US-EPA, 2006). Each sample was analyzed for As, Be, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sb, Se, Ti, Tl, V and Zn. We used Till 4 and Montana 2710 as standard reference materials for accuracy and

Why did you choose not to sample in annual-dominated areas?

performance checks of PXRF analysis. Values for elements that fell within the ± 20% values of the standard were taken as accurate.

Patterns of metal accumulation in plants. – The perennial species abundance survey was used to select the five most abundant species for analysis of metal accumulation. Tailing samples (200 g) were collected from the rhizosphere and 5-10 leaves were randomly collected from 6 individuals of each species distributed in at least two patches. Leaf tissue was rinsed with distilled water several times in the field and transported to the lab. Once in the lab, leaf tissue was rinsed with deionized water and dried at 50ºC for 48 h. Dried leaf tissues were ground in an agate mortar for analysis of metals. Metals in tailing material from the rhizosphere and from leaves were analyzed as previously described (PXRF).

Why did you only sample in some patches - did this relate to the fact that some species only occurred in 2 patches?

Experiment on local adaptation in an annual and a perennial species. – In order to test whether a particular plant species (either annual or perennial) had physiologically adapted to the local tailings conditions, we set up a reciprocal short-term experiment in a shade house at the Instituto de Ecologia UNAM in Hermosillo, Sonora. First, seeds of *Prosopis velutina* (perennial) and *Amaranthus watsonii* (annual) were collected from individual plants (four *P. velutina* trees and ten *A. watsonii* plants) growing within the tailings deposit and growing in an off-site area close (< 100 m) to the deposit. Additionally, tailings and off-site soil samples (5 kg) were taken from each of the sites where seeds were collected. These samples (tailings and off-site soil) were used to fill plastic tubes (5 cm in diameter by 20 cm long); half of the tubes were filled with tailings and the other half with off-site soil. Each family (seeds derived from a single plant) from each site was grown in both tailings and off-site soil. Tubes received either five *A. watsonii* seeds or one *P. velutina* seed. Seven tubes per mother (family) for a total of 140 tubes were used for *A. watsonii*, whereas for *P. velutina*, from 7 to 40 tubes per mother for a total of 226 tubes were used. Tubes were regularly irrigated and the experiment lasted one month. The following parameters were recorded: emergence, height, number of leaves and total, root, shoot and leaf dry mass after 30 d after germination.

Statistical analysis. – Plant abundance is expressed as means and standard deviations of the number of perennial and annual individuals recorded within plots across the four patches. Histograms with the frequencies of different size (height) classes found for each species were used to describe the population structure and the pattern of regeneration of the most abundant perennial species (Silvertown & Charlesworth, 2007). Measured parameters including pH, EC, texture (sand frequency), and metal concentrations in patches with vegetation and areas without vegetation were compared using paired t-tests. Ratios of metal accumulation were calculated from the mean values found for individuals (leaves/rhizosphere) of all species. A χ^2 test was used to determine whether the ratios were significantly greater than 1. For local adaptation a two way ANOVA was used to evaluate whether there were significant differences in height, number of leaves and total, root, shoot and leaf dry mass due to origin of plants (tailing *vs*. soil) and growth media (tailing *vs*. soil). Local adaptation is inferred if the results show that plants collected from tailings grew better in tailings than in off-site soil and if plants collected from off-site grew better in off-site soil than in tailings. All statistical analyses were performed with JMP (SAS Institute, 1997).

RESULTS

Plant inventory. - We recorded a total of 42 species of plants distributed in 16 families of angiosperms (Table 1). The most common families were Poaceae and Asteraceae.

Abundance and population structure. – A total of 872 individuals of perennial species were recorded growing in four patches in the center tailing. The five most common perennial species were, in order of decreasing abundance: *Acacia farnesiana* (N=540), *Brickellia coulteri* (N=126), *Gnaphalium leucocephalum* (N=108), *Baccharis sarothroides* (N=81) and *Prosopis velutina* (N=17). The abundance (number of individuals/100 m^2) varied among patches with values between 0 and 13 individuals/100 m^2 (Fig 1). Among the perennial species, three patterns of population structure were observed: a) species that are actively regenerating with a large number of the small size classes (seedlings) like *A. farnesiana* (Fig 2); b) species with large number of intermediate size classes (juvenile and adults), like *B. coulteri*, *B. sarothroides* and *G. leucocephalum* that are still regenerating (Fig 2) and c) species with no evidence of recent regeneration like *P. velutina* and composed mainly of large size (adults) classes (Fig 2).

The most common annual species were *Amaranthus watsonii*, *Boerhavia coulteri*, *Solanum holtzianum* and *Bromus catharticus* (Fig 1). The abundance varied among patches with values between 0 and 16 individuals/m^2.

I'm interested in why you chose to census the number of plants rather than looking at total vegetative cover. It might be fair to assume that the two are roughly correlated but it may also depend on what the dominant species is. Might it be interesting to look at commonly-used indicators of ecological function such as total cover, or the cover of different plant functional groups?

Relation between plant distribution and physicochemical properties of tailings. – There were no significant differences in measured physicochemical properties between samples taken from patches with vegetation and adjacent areas with no vegetation (pH, EC, texture, and metal content) (Table 2).

Patterns of metal accumulation in plants. - The accumulation factor (leaf/rhizosphere) for most metals in the five most abundant plant species was below 1 (Table 3). The exceptions were for Zn in *B. sarothroides* and *G. leucocephalum* where ratios of the accumulation factors were significantly greater than 1. Similarly, for most metals and plant species, leaf metal concentrations did not exceed domestic animal toxicity limits (Appendix 1). For Cu, all plant species reached the domestic animal toxicity limit (15 mg/kg for sheep and 40 mg/kg for cattle; Natural Research Council, 2005). Similarly, for Mo all plant species also reached the toxicity limit (5 mg/kg for cattle). In addition, for some species and metals, values approached the upper limit of the maximum tolerable range as Zn for *G. leucocephalum* and Ca for *P. velutina* (Appendix 1).

Experiment on local adaptation in an annual and a perennial species. -

~~Two of the plant species, *A. watsonii* and *P. velutina*, were tested for local adaptation (ecotypes).~~ For *A. watsonii*, seedling emergence varied between 30% and 44% among treatments. After emergence, seedling survival varied between 83% and 90% among treatments.

Would it not be more normal to report the mean and standard deviation?

Seedlings growing in tailing material reached 2.8-3.1 cm in height after 30 d whereas seedlings growing in off-site soil reached 7.4-8.2 cm (Fig 3A). The statistical analysis

Statistical results should be reported in full - F, p and degrees of freedom

revealed a significant difference due to growth medium ($F=442.8$, $p < 0.0001$) but no significant difference due to seed origin ($F=0.79$, $p = 0.3$). The mean number of leaves from seedlings growing in tailing material was 2 whereas seedlings growing in soil had 7 leaves (Fig 3B). As for growth, the statistical analysis indicates a significant difference due to growth medium ($F=369.1$, $p < 0.0001$) but no significant difference due to seed origin ($F=0.009$, $p=0.92$). For total dry mass, seedlings growing in tailing material accumulated 0.005-0.007 g and seedlings growing in soil accumulated 0.06-0.08 g. In this case, the analysis shows significant differences due to the growth medium ($F=193.8$, $p< 0.0001$). In addition, seedlings grew significantly better in the medium where they came from ($F=10.4$, $p < 0.01$) (Fig. 3C). Did you include an interaction between origin and medium?

For *P. velutina*, seedling emergence ranged from 67 to 98% among treatments. After emergence, seedling survival varied between 57 to 88% among treatments. After 30 d growth, seedlings growing in tailing material reached 4.5-4.6 cm whereas seedlings growing in off-site soil reached 8.8-9 cm (Fig 4A). The statistical analysis indicates a significant difference due to growth medium ($F=519.3$, $p < 0.0001$) but no significant difference due to seed origin ($F=0.98$, $p=0.3$). For the number of leaves, seedlings growing in tailing material had 3.1-3.7 leaves whereas seedlings growing in off-site soil had 4.6-7.5 (Fig 4B). In this case, a significant difference was detected due to growth medium ($F=55.5$, $p < 0.0001$) and given that seedlings derived from seeds from off-site soil grew better in both media, a significant difference was also detected due to seed origin. For total dry mass, seedlings growing in tailings accumulated 0.019-0.02 g whereas seedlings growing in off-site soil accumulated 0.06-0.07 g (Fig 4C). As for number of leaves, a significant difference in dry mass was detected due to growth medium ($F=193.8$, $p < 0.0001$) and also due to seed origin ($F=10.4$, $p=0.0013$), given that seedlings coming from the off-site area grew better than seedlings coming from tailings.

I think you'd be better off reporting your ANOVA results in a table that included the main effects and interaction terms and the full results (F, p and d.f.)

DISCUSSION

Successful implementation of phytostabilization on mine tailings, the establishment of a permanent vegetative cover, requires careful consideration of the plants to be used. This ideally includes a combination of different perennial species with different rooting depths and

canopy cover structure that do not accumulate metals into shoot tissues (Mendez & Maier, 2008). In addition, the geographic range of plant species must be taken in consideration, making plants that can naturally colonize mine tailings of interest for their phytostabilization potential. This study identified 42 different species of plants growing in small patches on the abandoned Nacozari mine tailings. Fifteen of these species were perennial whereas twenty seven were annual. From the set of fifteen perennial species, only five were abundant and from this set, four of the five showed clear evidence of regeneration, i.e., the ability to reproduce and establish new seedlings. This set of perennial species normally produces seeds almost every year (Molina-Freaner, pers. obs.) but recent seedling establishment is restricted to *A. farnesiana*, *B. coulteri*, *B. sarothroides* and *G. leucocephalum*. We do not know the mechanism that restricts recent seedling establishment in *P. velutina* and future studies should
What are your hypotheses and how would you proceed with this question?
identify the regeneration barriers of perennial species in this tailings. Taken together, these results suggest that this set of species has potential for phytostabilization of the Nacozari tailings, as they include trees (*P. velutina* and *A. farnesiana*) with relatively large canopy cover and deep roots, and shrubs (*B. coulteri*, *B. sarothroides* and *G. leucocephalum*) with smaller cover and relatively shallower roots.

Annual species identified on this site had greater diversity (27/42) than the perennial species (15/42). However, annual species have a more limited potential for phytostabilization given their shallow roots, short life cycles and the fact that they grow only during the summer rainy season in this region (Shreve & Wiggins, 1964). However, they may regularly add organic matter to the tailings facilitating the establishment of perennial species.

Identifying factors that limit plant establishment in tailings is an important goal in phytostabilization (Parrado-Aguado et al., 2013). This study recorded only four patches of vegetation on the tailings, representing 0.84% of the 19 ha surface area. These patches which contained 872 individuals of perennial species have very slowly colonized the tailings over more than 6 decades since the mine was closed in 1949. This suggests there are key inhibitory factors preventing seed germination and seedling establishment at this site. Paired sampling comparing patches of vegetation with adjacent barren areas revealed no differences in pH, electrical conductivity, texture or the concentration of potentially toxic elements. So these factors do not influence the distribution of plants in the four patches at this site. Future studies should explore whether organic matter, major nutrients (such as N or P), moisture content, or the composition of microbial communities restrict plant establishment in this site to these four small patches.

It could also just be a stochastic process mediated by the need for regeneration microsites and the distance from nearby seed sources. The latter is a major barrier. Would it be interesting to examine i) seed dispersal and distribution across the site (i.e. seed rain and seed bank structure); and ii) processes of competition and facilitation that might control vegetation establishment and community assembly following initial colonization?

Plants used in phytostabilization should be metal tolerant but limit metal accumulation into above ground tissues in order to avoid transferring toxic elements into food chains (Mendez & Maier, 2008). The five most abundant plant species from the Nacozari tailings had metal accumulation ratios that were generally below 1. The exception was Zn for which two perennial species had accumulation factors greater than 1. Thus, of the five most abundant perennial species, three comply with the requirement of low accumulation. In addition, plants suitable for phytostabilization should have metal concentration in their leaves below the maximum tolerable level for animals (Mendez & Maier, 2008). In our study, we recorded that the five most abundant perennial species have Cu and Mo concentration in their leaves above animal toxicity levels (Natural Research Council, 2005). Given the accumulation of Cu, Mo and Zn in the most abundant species, future studies should explore whether amendments such as compost, could reduce metal accumulation and meet requirements for animal toxicity (Solis-Dominguez et al., 2012).

The success of phytostabilization depends on the use of locally adapted plants. It has been previously suggested that using locally adapted ecotypes provides better cover and persistence than other sources of seeds when grown in mine wastes (Smith & Bradshaw, 1979). Our short-term experiments testing local adaptation in an annual and a perennial species did not detect strong evidence of tailings ecotypes even after a period of more than 60 years. Our data reveal that tailings as a growth substrate significantly reduced the amount of biomass produced when compared with plants grown in off-site soil. However, both the annual and perennial species tested show that seeds harvested from plants growing on the tailings site or in a neighboring off-site area are equally effective for phytostabilization in the Nacozari tailings. Thus, we suggest that future field studies in the Nacozari tailings should use local sources of seeds.

I might tend to disagree. With no obvious benefit of using seeds from plants on the tailings I would suggest that ensuring genetic diversity at the site by using regionally-adapted seed will be more important. Only using seed from the site itself could lead a genetic bottleneck and reduce overall fitness and resilience in the future.

In conclusion, this plant inventory identified several native perennial species with potential for phytostabilization of the Nacozari mine tailings. One advantage of using these plants is that seed could be collected locally and used to revegetate the site. However, two of the species identified accumulate Zn and all species accumulate Cu and Mo into the range of maximum tolerable levels for animals (National Research Council, 2005). Thus, measures should be taken to evaluate and minimize the risk of transferring toxic elements into the local food chain.

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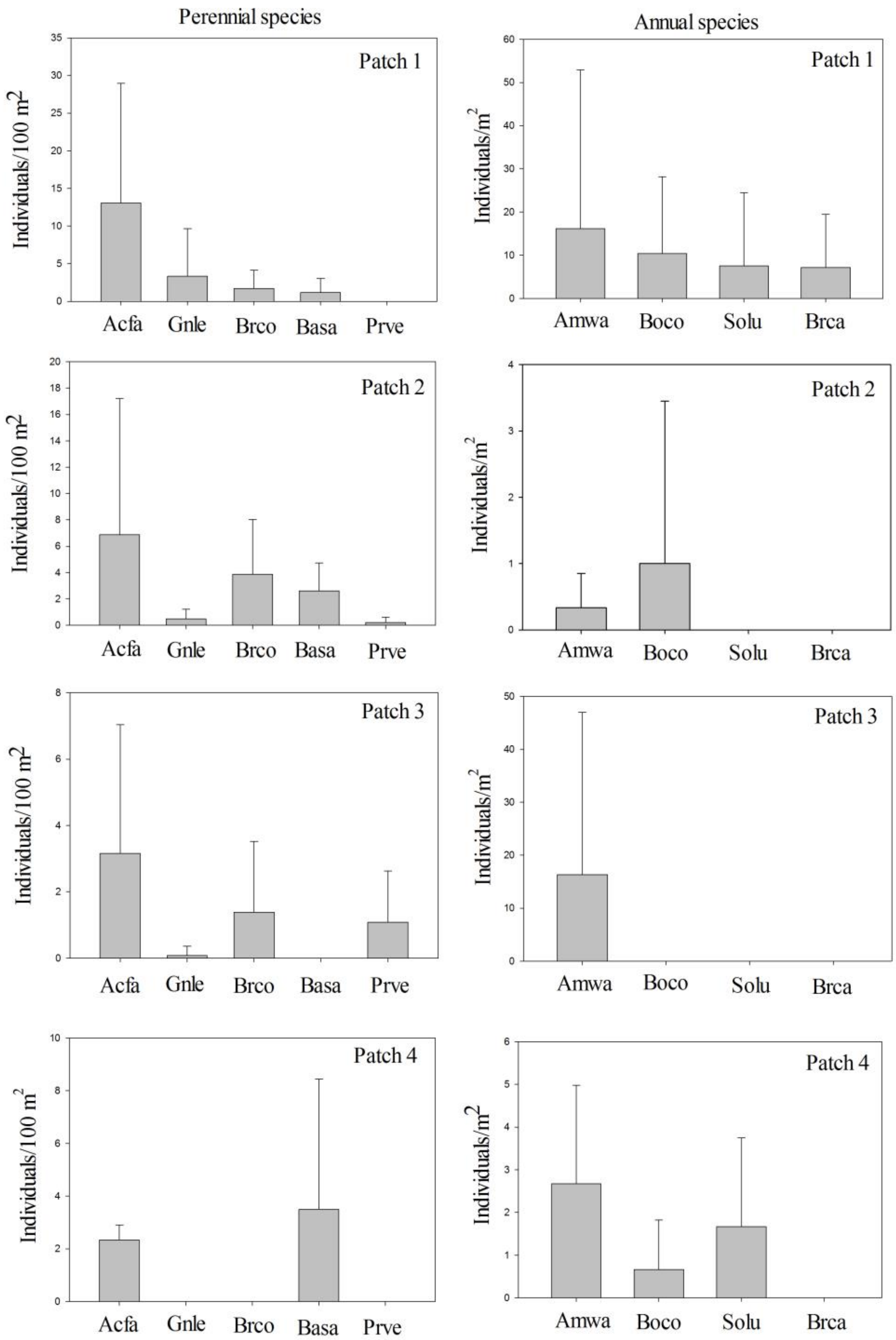
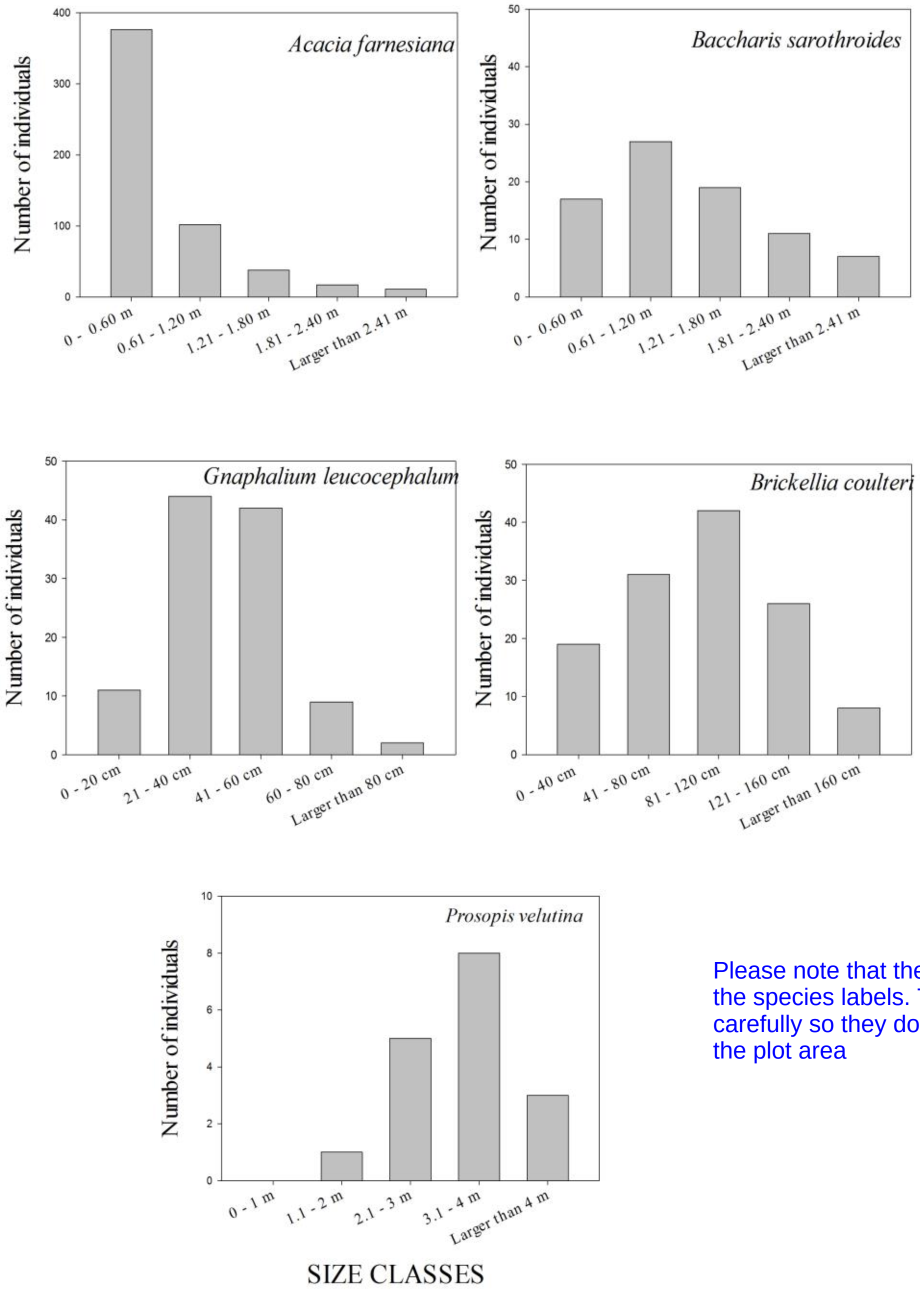


I would prefer to see data like this presented as a box plot as it better represents the variation in abundance. At the very least error bars should be defined (stanadard error or standard deviation?) and placed above and below the mean

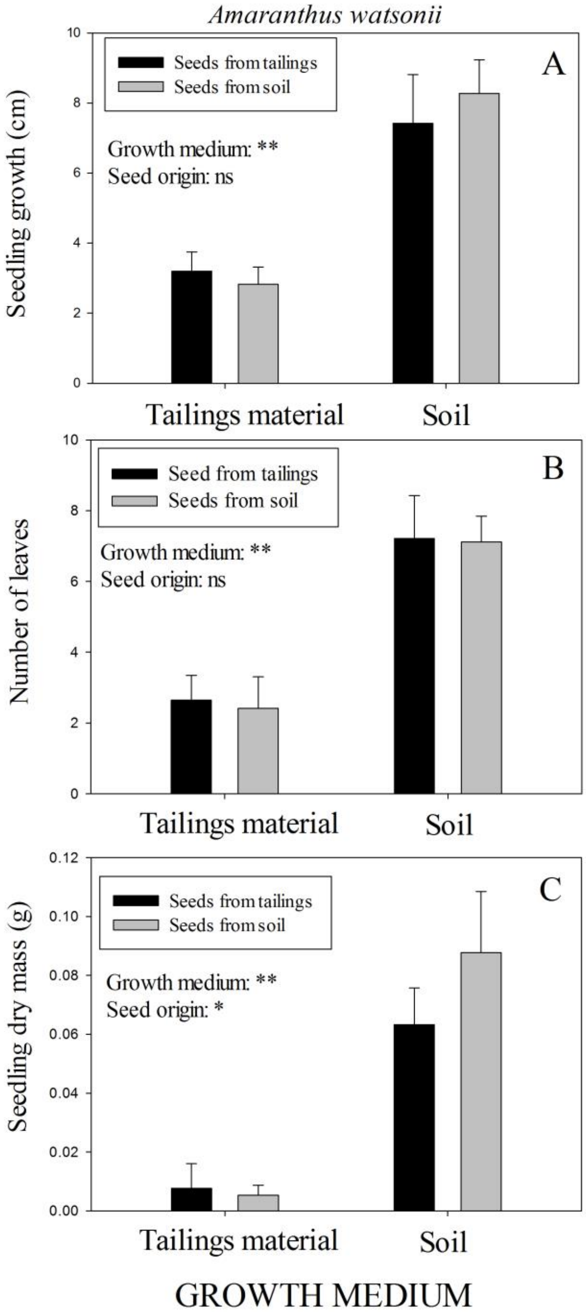
Figure 1. - Abundance of perennial and annual species recorded at four patches in the center Nacozari tailings deposit. Perennial species; Acfa: *Acacia farnesiana*, Gnle: *Gnaphalium leucocephalum*, Brco: *Brickellia coulteri*, Basa*: Baccharis sarothroides* and Prve: *Prosopis velutina*. Annual species; Amwa: *Amaranthus watsonii*, Boco: *Boerhavia coulteri*, Solu: *Solanum lumholtzianum* and Brca: *Bromus catharticus.* Notice that unit area is 100 m^2 for perennial species and 1 m^2 for annual species.



Please note that there are differences in font size in the species labels. These also need placing more carefully so they don't extend beyond the margin of the plot area

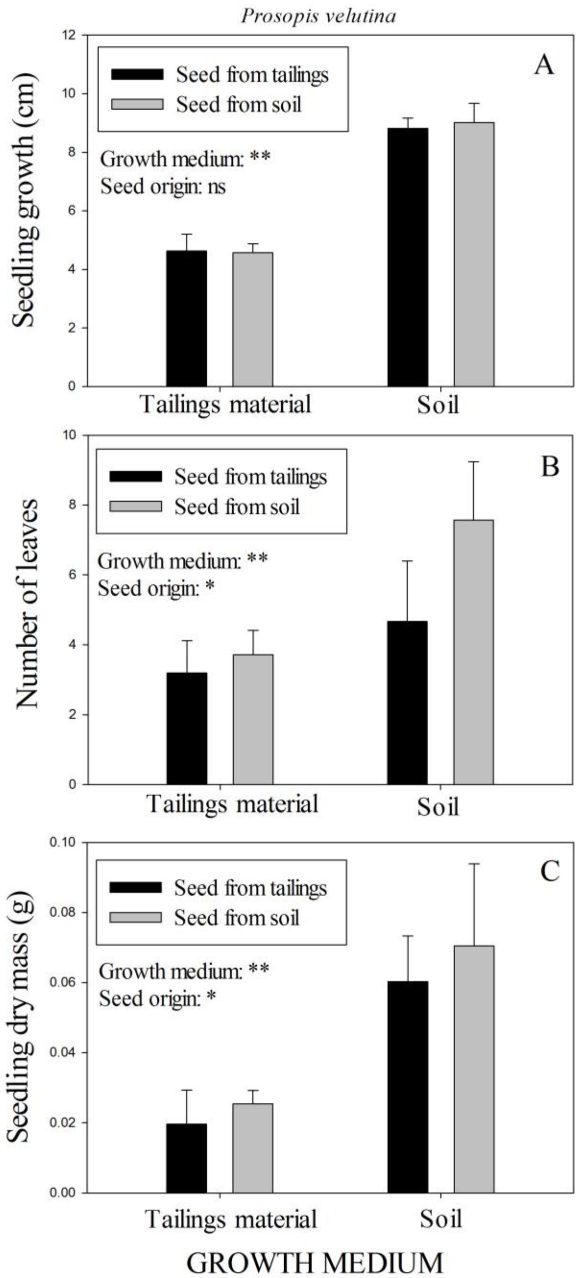
Presumably this is data combined across all four different patches? Would it be interesting to use stacked bar charts to examine whether there are differences in population structure between the patches?

Figure 2. - Population structure of the most common perennial species recorded at the center Nacozari tailings deposit: *Acacia farnesiana* (n= 540 individuals), *Brickellia coulteri* (n= 126 individuals), *Baccharis sarothroides* (n= 81 individuals), *Gnaphalium leucocephalum* (n= 108 individuals), and *Prosopis velutina* (n= 17 individuals).



Please see earlier comment about error bars

Figure 3. – Height (A), number of leaves (B) and total dry mass (C) of seedlings of *Amaranthus watsonii*coming from two different sources (tailings and an adjacent site with normal soil) after 30 days of growth in tailing material and soil. Significant differences due to growth medium or seed origin are indicated with asterisks



Figures 3 and 4 could be combined into a single 3x2 figure

Figure 4. – Height (A), number of leaves (B) and total dry mass (C) of seedlings of *Prosopis velutina* from two different sources (tailings and adjacent site with normal soil) after 30 days of growth in tailing material and soil. Significant differences due to growth medium or seed origin are indicated with asterisks.

Table 1. - List of species recorded at the center tailing of Nacozari, Sonora. (√: Present; x: absent)

Scientific names should be in italics. I think this inventory would be more appropriate in an Appendix

Family	Scientific name	Patch 1	Patch 2	Patch 3	Patch 4
Amaranthaceae	Amaranthus watsonii	√	√	√	√
Amaranthaceae	Chenopodium neomexicanum	√	x	√	x
Amaranthaceae	Ambrosia confertiflora	√	√	√	x
Asteraceae	Ambrosia ambrosioides	x	√	x	x
Asteraceae	Baccharis sarathroides	√	√	x	√
Asteraceae	Brickellia coulteri	√	√	√	x
Asteraceae	Gnaphalium leucocephalum	√	√	√	x
Asteraceae	Machaeranthera tagetina	√	x	x	x
Asteraceae	Machaeranthera gracilis	√	x	x	√
Cannabaceae	Celtis pallida	√	√	x	x
Commelinaceae	Commelina erecta	√	x	x	√

Convolvulaceae	Ipomoea arborescens	x	x	√	x
Convolvulaceae	Ipomoea cristulata	x	x	√	x
Convolvulaceae	Ipomoea purpurea	x	x	√	x
Cucurbitaceae	Cucurbita digitata	√	x	√	x
Cyperaceae	Cyperus elegans	x	x	x	√
Euphorbiaceae	Euphorbia heterophylla	√	x	x	x
Euphorbiaceae	Chamaesyce albomarginata	√	x	x	x
Euphorbiaceae	Ricinus communis	x	x	√	x
Fabaceae	Acacia farnesiana	√	√	√	√
Fabaceae	Prosopis velutina	x	√	√	x
Malvacea	Sida rhombifolia	√	x	x	x
Molluginaceae	Mollugo verticillata	x	x	√	√
Nyctaginaceae	Boerhavia coulteri	√	√	√	√
Poaceae	Bouteloua repens	√	x	√	x

Poaceae	Bromus catharticus	√	x	x	x
Poaceae	Cenchrus ciliaris	x	√	x	x
Poaceae	Chloris virgata	x	√	√	X
Poaceae	Cynodon dactylon	√	√	√	x
Poaceae	Echinochloa colonum	x	x	x	√
Poaceae	Eragrostis lugens	x	x	√	x
Poaceae	Panicum hirticaule	√	x	√	x
Poaceae	Setaria macrostachya	√	√	√	X
Poaceae	Sorghum halepense	x	√	√	√
Poaceae	Urochloa arizonica	√	x	x	x
Portulacaeae	Portulaca suffrutescens	√	x	x	√
Portulacaeae	Talinum paniculatum	x	√	x	x
Rhamnaceae	Ceanothus greggii	x	√	x	x
Solanaceae	Datura wrightii	√	√	x	x
Solanaceae	Nicotiana glauca	x	x	√	x

Solanaceae	Solanum elaeagnifolium	√	x	x	√
Solanaceae	Solanum lumholtzianum	√	x	x	√

Table 2. -Physicochemical properties (mean ± standard deviation) of the center Nacozari tailing from patches with vegetation and adjacent areas with no vegetation.

Property	Patch with vegetation	Area with no vegetation	Statistical test	Significance
pH	4.7 ± 0.2	4.5 ± 0.3	t= 0.97	p=0.18
Electrical conductivity (µS/cm)	162.3 ± 80.1	112.9 ± 10.3	t= 1.06	p=0.19
Percentage of sand	77.8 ± 4.5	79.8 ± 3.7	t= 0.57	p= 0.30
Percentage of clay	5.7 ± 2.9	6.1 ± 2.0	t= 0.19	p=0.57
As (mg kg^{-1})	18.7 ± 2.3	21.0 ± 1.9	t=0.88	p=0.79
Ba(mg kg^{-1})	1,091.5 ± 46.8	1,172.5 ± 76.0	t=1.81	p=0.06
Ca(mg kg^{-1})	2,872.7 ± 1,779.1	1,286.0 ± 926.8	t=2.07	p=0.06
Cu(mg kg^{-1})	333.0 ± 96.3	271.5 ± 45.7	t=0.05	p=0.47
Fe(mg kg^{-1})	26,167.7 ± 6,454.6	31,604.2 ± 7,273.2	t= 1.32	p=0.88
K (mg kg^{-1})	35,741.2 ± 1,247.6	36,821.0 ± 1,557.6	t=2.25	p=0.03
Mn(mg kg^{-1})	224.0 ± 22.7	225.7 ± 11.9	t=0.81	p=0.77
Mo(mg kg^{-1})	57.2 ± 15.8	65.5 ± 9.1	t=1.54	p=0.90
Pb(mg kg^{-1})	32.7 ± 6.5	34.0 ± 8.7	t=0.26	p=0.59
Rb(mg kg^{-1})	259.0 ± 9.1	255.0 ± 7.0	t=0.43	p=0.34
Sr(mg kg^{-1})	94.2 ± 22.5	84.5 ± 21.4	t=1.85	p=0.07
Ti (mg kg^{-1})	1,536.0 ± 208.7	1,394.5 ± 176.4	t=1.26	p=0.12
Zn(mg kg^{-1})	69.0 ± 3.4	68.7 ± 1.9	t=0.64	p=0.27
Zr(mg kg^{-1})	142.7 ± 4.8	136.2 ± 3.6	t=1.83	p=0.06

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Table 3. –Metal accumulation factors (mean ± standard deviation) of the most abundant perennial species in the center tailing of Nacozari. Accumulation factors are calculated as element concentration in leaves/element concentration in the rhizosphere.

Plant species / **Element**	*Baccharis sarotroides*	*Gnaphalium leucocephalum*	*Brickellia coulteri*	*Acacia farnesiana*	*Prosopis velutina*
Cu	0.26 ± 0.10	0.8 ± 0.31	0.22 ± 0.08	0.44 ± 0.15	0.51 ± 0.24
Fe	0.006 ± 0.002	0.13 ± 0.06	0.01 ± 0.005	0.04 ± 0.15	0.05 ± 0.02
K	1.32 ± 0.43	2.35 ± 0.56	1.57 ± 0.30	0.68 ± 0.16	0.63 ± 0.32
Mn	0	3.44 ± 2.40	1.25 ± 0.65	0	0
Mo	0.23 ± 0.06	0.27 ± 0.08	0.19 ± 0.05	0.26 ± 0.06	0.22 ± 0.03
Rb	0.24 ± 0.08	0.38 ± 0.23	0.21 ± 0.07	0.21 ± 0.03	0.17 ± 0.04
Sr	0.58 ± 0.62	0.52 ± 0.33	0.51 ± 0.13	0.97 ± 1.0	2.20 ± 1.9
Zn	3.45 ± 3.6 *	9.11 ± 8.5 *	1.7 ± 2.6	1.6 ± 1.9	1.55 ± 2.8
Zr	0.06 ± 0.03	0.11 ± 0.03	0.05 ± 0.006	0.07 ± 0.005	0.064 ± 0.008

Mean ratios (leaves/rhizosphere) for all species were evaluated using a χ^2 test. Ratios that were significantly greater than 1 are indicated with an asterisk (*).

Appendix 1. – Mean metal concentration (mg/kg) in leaves of the most abundant perennial species from the center Nacozari tailings deposit.

Element	Plant species					Maximum Tolerable Level for Animals[2]
	Baccharis sarothroides	*Gnaphalium leucocephalum*	*Brickellia coulteri*	*Acacia farnesiana*	*Prosopis velutina*	
Ca	17260± 7653	17029± 2957	21552± 2577	25795± 12737	49225 ± 13333	0.9 – 2% dry mass
Cu	71.43± 15.0	279.49± 91.96	60.04± 13.64	124.33± 28.94	208.50± 48.73	15 – 500 mg/kg
Fe	178.47±69.04	4456 ± 1440	429.76± 270.88	1148± 1182	1708±442	500 – 3000 mg/kg
K	44976± 14042	73824± 6073	53581± 7377	24631± 4563	21899± 10608	1-2% dry mass
Mn	<BDL[1]	857.9± 550.4	320.17± 180.03	<BDL	<BDL	400 – 2000 mg/kg
Mo	11.11± 0.83	17.41± 1.15	10.76± 0.95	13.88± 1.47	14.29±1.32	5 – 150 mg/kg
Rb	59.23± 22.23	80.28± 35.13	51.51± 19.13	53.77± 7.16	47.30±12.59	200 mg/kg
Sr	52.47± 35.72	54.13± 13.96	50.50± 18.34	80.32± 68.97	194.9±135.7	1000-2000 mg/kg
Zn	277.2 ± 209.1	807.1 ±460.1	258.49± 157.72	159.7 ± 117.79	342.74±164.47	250-1000 mg/kg
Zr	10.46± 3.61	16.08± 3.89	8.17± 0.67	10.17± 0.85	9.16±1.20	n.a.[3]

[1]BDL= below detection limit

[2]Ranges represent values from the NRC (2005) report "Mineral Tolerance of Animals". The maximum tolerable level is defined in the report as "the dietary level that, when fed for a defined period of time, will not impair animal health and performance". Ranges are provided since values differ for animals tested including swine, poultry, horses, cattle, sheep and fish.

[3]n.a. = not available

Mote that there are some formatting errors and missing spaces in this table.