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Evaluation of Uranium mine tailing remediation by amending land soil and invading native plant species

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Abstract: *Properties of the mine waste represented the toxic nature to human health and may pose numerous risks to the local environment. Although the recorded radioactivity level in these tailings is very low, but to avoid any long term effect of these tailings on the atmosphere as well as native living things, the tailings need to cover with soil. This reduces gamma radiation and radon emission levels. However, to consolidate the radioactivity and remediate the contaminants in the tailings on a sustainable basis, the area needs to be re-vegetated by candidate plants. Remediation of heavy metal-contaminated sites using plants presents a promising alternative to current methodologies. Therefore, the study was to evaluate the ex-situ phyto-remediation of uranium mine tailing ponds by amending with land soil followed by invading to grow predominant native plant species. Three volumes of the pot culture experiment were carried out i.e. additions of land soil to mine tailing at 0, 50, and 100 percent by volume with four abundance plant species of* S. spontaneous (*terrestrial*), *P. vittata (fern) and T. latifolia and C. compressus (aquatic species) were selected in the study. After appropriate preparation U, Mn, Fe, V, Ni, Cu and Zn which were the major contaminants and the pH and EC in soil fractions and also growth parameters in plant materials were analyzed at five stage intervals in the duration of four months. The addition of soil was found significant change in pH to alleviate the toxic effects that heavy metals have on plant health, hence the enhanced growth and survivability was reached. Even while redistributing metals to a less available form, during the remediation the plant species have the capabilities to accumulate substantial amount of toxic metals. The metal concentrations in the plants were found in the order Fe50.92 > Mn7.22 > Zn0.94 > Cu0.92 > Ni0.65 > V0.18 > U0.07 and accumulated in the order Zn0.021 > Cu0.019 > Mn0.014 > Ni0.013 > U0.002 > V0.001 > Fe0.001. The results of the study indicate that C. compressus and S. spontanium were found to be the candidate species for Phyto-remediation (i.e. either accumulation and non-accumulation or consolidation respectively) of contaminants in soil amended Uranium mine tailings. For maximum accumulation, C. compressus harvesting need to be done at after the fourth month of plantation and for consolidation, S. spontanium plantation was greatly recommended. It also confirms that the land soil could be the best amendment for remediation of abandoned mine waste.*

Keywords*: Native plants; Phyto-remediation, Soil amendment, Transfer factor, Uranium tailing*

I. Introduction

Uranium mine tailings (UMT) are the crushed rock residues remaining following the extraction of uranium from ores. By virtue of the residual radioactivity associated with decay products of uranium, such tailings constitute a high-volume, low-level radioactive waste. Disposal of mine tailing wastes by landfill in the form of slurry is the most widely practiced method in the world including India. Therefore, radionuclide and metal pollution is a global environmental problem and the number of contaminants entering the environment has increased greatly in recent times due to increased mining activities [1]. Toxic heavy metal contamination is prevalent within the surface soils and water at mining and industrial sites [2]. Therefore, heavy metal pollution is a widespread problem within all industrially developed countries of the world. Past waste disposal practices associated with mining and manufacturing activities have been such that air, soil, and water contamination were common, and as a result there are many metal contaminated sites that pose serious health risks [3]. The fate of various metals, including chromium, nickel, copper, manganese, mercury, cadmium, and lead, and metalloids, including arsenic, antimony, selenium, and radionuclide's in the natural environment is of great concern [4][5], particularly not only near former mine sites, dumps, tailing piles, and impoundments, but also in urban areas and industrial centers. Therefore, there is a need for cost-effective, low energy technologies that can be applied at these sites.

In these circumstances, long-term stabilization of radionuclide's and other pollutants from contaminated substrates is a key-criteria for the success of rehabilitation works. In situ soil remediation at sites with low-levels of U contamination, involving the use of chemical stabilizing agents and subsequent re-vegetation, is attractive because it is expected to be more cost effective and less disruptive to the environment [6][7][8]. Chelates have been used in soils and nutrient solutions to increase the solubility of metal cat ions in plant growth media and are reported to have significant effects on metal accumulation in plants [9][2]. However, chelating agents are more expensive than compost amendments and require careful management [2], and the uranium mine tailing pond containing the waste in the form of crushed rock powder [10]; [11]. Therefore, synthetic or natural soil amendments are needed to decrease or increase of the availability of heavy metals thereby enhance plant up-take [12] [13].

Organic soil amendments can reduce metal toxicity to plants by redistributing the metals to less available fractions. Phosphates such as apatite amendments have been successfully used to lower the bioavailabitity and increase the geochemical stability of metal contaminated soil [14]. Compost and other amendments can be used as a vital tool to restrict the availability of heavy metals in soil [15] [16]. Organic amendments aid in a binding process that occurs if high pH levels are maintained. The addition of compost to decrease plant accumulation of heavy metals was chosen as an alternative to traditional soil amendments [16]. Organic soil amendments can ameliorate metal toxicity to plants by redistributing metals to less available fractions. The association of heavy metal with organic matter varies directly with soil pH [17]. The distribution of metals is significantly influenced by soil pH and organic matter content [18][19]. The up-take of many heavy metals can be increased by the reduction of pH within the soil. Heavy metal bioavailability increases with the decrease of pH. Decreasing pH will in turn increase the solubility of metals. [20] Reported the high pH of the compost affected the solubility of the metal hydroxide and carbonates, and low pH values increase the solubility of heavy metals. The pH of soil can be a direct determinant of the type of association heavy metals have with organic materials [21]. Organic amendments are used as an effective tool to reduce plant up-take if high soil pH levels are maintained [22].

Fig–1 illustrates how soil amendments can help mitigate exposure to contaminants. With the addition of appropriate soil amendments, metals in the amended area are chemically precipitated and/or sequestered by complexation and sorption mechanisms within the contaminated substrate. Metal availability to plants is minimized, and metal leaching into groundwater can be reduced. In certain cases, metal availability below the treated area is also reduced. Soil amendments can be used to address two primary categories of problems at contaminated sites: (1) contaminant bioavailability and phytoavailability; and (2) poor soil health and ecosystem function [23]. The addition of amendments restores soil quality by balancing pH, adding organic matter, increasing water holding capacity, re-establishing microbial communities, and alleviating compaction. As such, the use of soil amendments enables site remediation, revegetation and revitalization, and reuse. Apart from that, amendments such as compost and chelating agents are used to separate heavy metal from soil particles, which increases the metal's availability to the plants species within the soil. However, long-term effects of chelates within soil have been found to be detrimental to the environment and the addition of these amendments release heavy metals, which bind to soil particles. Heavy metals bind to the surface of soil particles, resulting in a reduction of availability [23]. The release of these heavy metals results in the increase their availability, which will additionally increases the risk of metals leaching into the surrounding water system. For the reason, using only the land soil as an amendment can aid in the growth and surveillance of plant species and also play the vital role in the accumulation efficiency of toxic contaminants by the selected plant species [13]. A wide variety of soil amendments are used to increase and lower the availability of heavy metals in Phyto-remediation procedures. The use of plants to remove hazardous heavy metal contamination from soil is a promising alternative method of soil remediation worldwide.

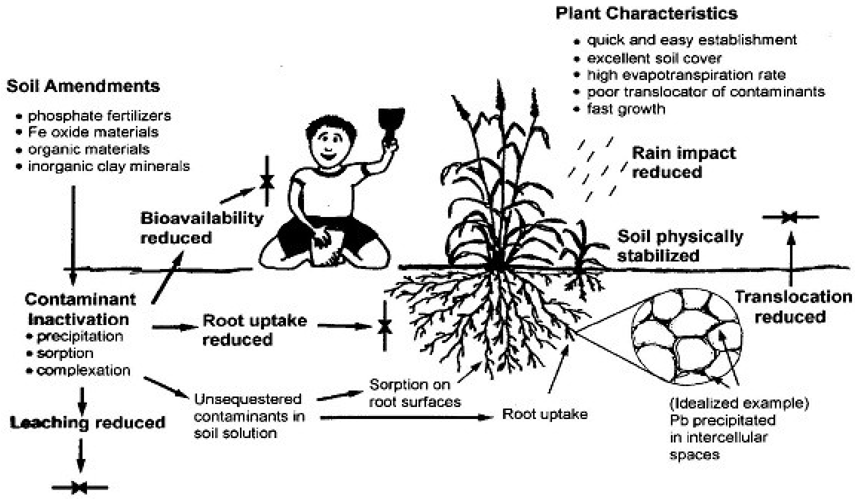


Figure – 1: The Role of Soil Amendments and Plants in the Metal-Contaminated Soil [24].

Figure – 2: Uranium mine tailing ponds at Jharkhand state: A. Turamdih Tailing Pond, B. JadugudaTailing Pond.

UMT is the crushed rock residues of the uranium extraction process from ores. By virtue of the residual radioactivity associated with decay products of uranium, such tailings constitute a high-volume, low-level radioactive waste [11]. The tailings effluent and tailings solids from the mill are discharged as slurry to a waste retention pond, called tailing pond. Natural radionuclides' and trace metals are present in mine tailing/soil in varying concentrations, some of these are found in elevated concentrations in uranium waste tailings. In India uranium mine tailing ponds at Jaduguda and Turamdih receives waste from ores mined at the six mine stations in Jharkhand (Fig–2). Although the recorded radioactivity level in these tailings is very low, but to avoid any long term effect of these tailings on the atmosphere, human ,cattle as well as native flora and fauna, the tailings have been covered with 30cm. layer of soil. This reduces gamma radiation and radon emission levels. However, to consolidate the radioactivity in the tailings on a sustainable basis, the area needs to be revegetated by selecting plant species, having shallow root systems; good conservation value and low canopy cover [25].

Current remediation technologies fall into four basic categories, namely: physical, chemical, biological and thermal. Thermal treatment will remove contaminants, but will destroy other soil characteristics beneficial for plant growth. Chemical extraction and soil washing can also degrade the quality of the soil. To study the restoration of contaminated soils or degraded land, the effect of amending a land soil and to bioremediated soils with green waste compost and their subsequent ability to support the growth of grass and trees has been assessed [26]. Phyto-remediation is a natural process carried out by plants that are able to live in a contaminated media. Hyperaccumulators are plants that can absorb high levels of contaminants with their roots and concentrate them either within their roots, shoots, and leaves [27]. A variety of plant species are commonly used to remove heavy metal from the soil. The use of soil amendments can be a cost-effective in situ process for remediation, revitalization and reuse [28].

Soil amendments can be used to address contaminant bioavailability and phytoavailability; and poor soil health and ecosystem function. Soil amendments can reduce the bioavailability of a wide range of contaminants while simultaneously enhancing re-vegetation success and thereby protecting against off-site movement of contaminants by wind and water [29]. Using land soil as tailing amendments offer the potential for significant cost savings compared to traditional alternatives [29]. In addition, land revitalization using soil amendments can provide significant ecological and community benefits, including wildlife habitat, species diversity, food control, aesthetics and recreation [12][28]. Recent vegetation programs on disturbances have begun to emphasize the use of native vegetation. [30] Made an attempt to revegetate rock phosphate mine with various native trees, shrubs and grasses. They reported that the mixture of natives has improved the soil fertility status and productivity capacity of the spoil besides favoring the biological invasion of various natural invaders. [31] Emphasized that native species were less competitive and can be used in rehabilitation and the disturbances permits the germination and development of non-seeded species. The development of the ecosystem was accompanied by improvement in soil characteristics. Some species play a key role in nutrient conservation and were thus important in any rehabilitation program.

Therefore the present study is to evaluate the efficiency of land soil as effective tailing amendment within the heavy metal and radionuclide's contaminated uranium mine tailing ponds. And also to determine the hyper accumulation of known contaminants [Uranium (U), Manganese (Mn), Iron (Fe), Aluminum (Al), Vanedium (V), Nickel (Ni), Cupper (Cu), Zinc (Zn), Cobalt (Co) and Selenium (Se)] in the tissue systems of native plant species: S. spontaneous (terrestrial), P. vittata (fern) and T. latifolia and C. compressus (aquatic) species. This paper provides information on the use of soil amendments, a cost effective process for phyto-remediation, revitalization, and reuse of many types of disturbed and contaminated landscapes. Ex-situ experiments carryout to validate, understand and quantitate scientific parameters in the remediation process also formed a subject matter of this project.

II. Materials And Methods

2.1. Site description:

In India, Jaduguda Uranium mine is located at east longitude of 86^0 20' and north latitude of 22^0 40' and Turamdih Uranium mine is located at east longitude of 86^0 09' and north latitude of 22^0 43' at 24 km east and 5 Km south of Tatanagar railway station, in Jharkhand State, India [32]. The details of mining and processing technique are being described elsewhere [33] [34]. In the course of mining to milling, bulk of the ore processed emerges as tailings (residues from ore processing) and are pumped into a tailing pond. There are three valley-dam types of TPs at Jaduguda and fourth at Turamdih (Fig–2 & 3) (22°39'17.90"N and 86°19'51.14"E Google Earth; [35].

The first and second stages of the TPs are located adjacent to each other in a valley with hills on three sides and engineered embankments on downstream side of natural drainage [36]. These two TPs are filled up and now left abandoned and second stage TP was completely buried with 30cm thick land soil on the top [13]. The third and fourth stage of the TPs which is currently in use is also located nearby in a similar setting. These two active TPs are filled with effluent obtained after the ion exchange process of uranium removal and the fine particles obtained after the secondary filtration of barren liquor. The precipitates settle down in the TP and the clear liquid is continuous to decant from abandoned (closed) and active uranium mine TPs through a series of decantation wells and the decanted effluent has subsequently been manifested at various stages to treat through effluent treatment plant (ETP). The treated AMD found its way into an adjoining natural water source through Gala river and flowed towards downstream and finally mixing into Suwarnarekha river [37][38].

2.2. Collection of Tailing, Amendment and selection of Contaminants and Plant spp.

In the present study, two open landfills at Jaduguda and Turamdih tailing ponds (JTP & TTP) sites were selected for collection of mine tailing, seedling and sampling. Seedlings of plant materials and mine tailing have been done from each study site with the permission of the authorities. Four plant species (T. latifolia, S. spontaneous, P. vittata and C. compressus) were selected for the investigating the pot culture Phyto-remediation experiment (Fig–4). Plant species selection was based on having shallow rooted, are easy to adapt, good conservation value and also considering the species abundance, growth, harvest, nutrient assimilation and tolerance potential [13][25]. Apart from the above, the plants were chosen because the easily available and mainly these were non-edible to even the cattle. Amended soil used in this study was a freely available uncontaminated land soil. The major tailing contaminants of seven elements – Aluminum , Manganese, Iron, Nickel, Copper, Zinc and Uranium were chosen for phytoremediation in this study [39].

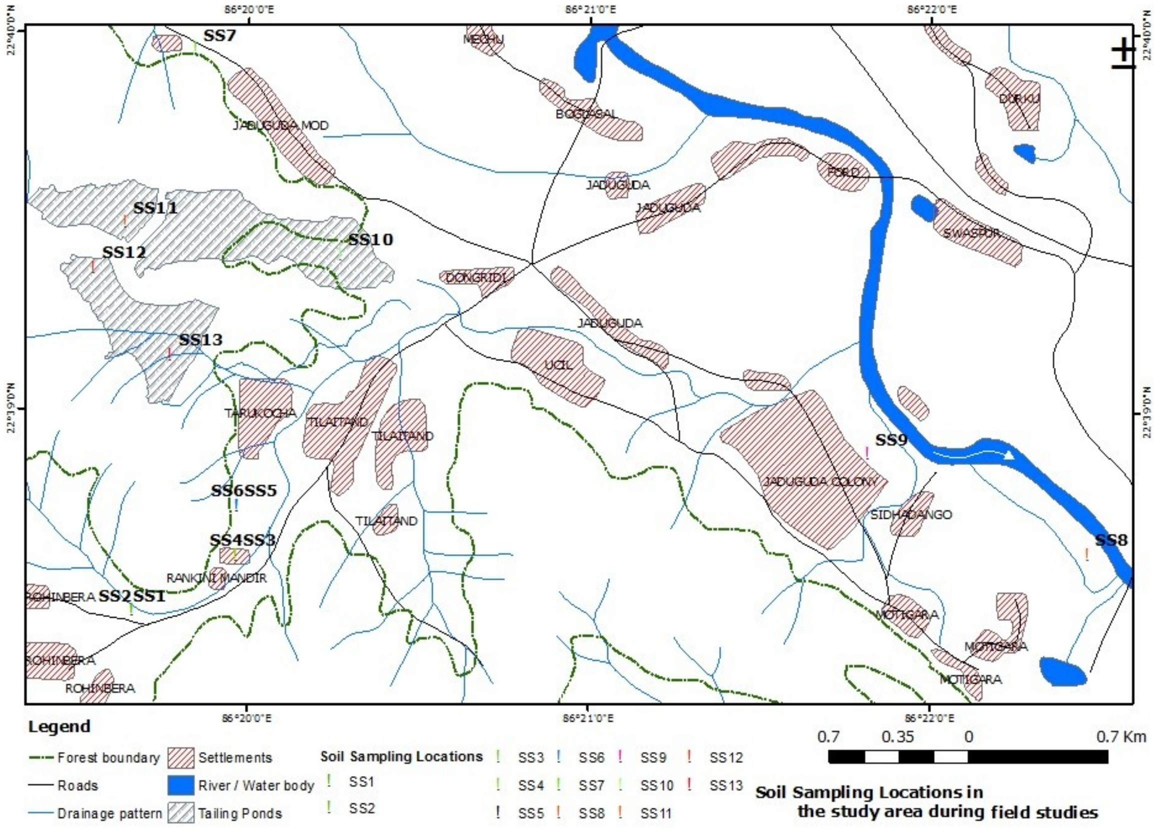


Figure – 3: Study site and sampling location map of Jaduguda tailing ponds and control. The map of Turamdih tailing pond is not presented here as it is also in similar setting.

Figure – 4: Selected plant species for experimental study

2.3. Experimental setup and preparation of test pots

The experiment was set up at the Environment Protection Training and Research Institute (EPTRI), Hyderabad (A.P.) India. All the experiment was run in the controlled poly-house condition to support the plant growth and survival at the initial phase of Phyto-remediation. Before the pot-culture prepared, the tailing and soil were air dried, sieved with a three mm plastic sieve and mixed thoroughly. 300cc root trainer and 5 kg pots were selected for short and long period experiment and filled using the uranium mine tailing which was amended with land soil at three concentration level i.e. T1: (100% or crude mine tailing), T2: (50% or half mix tailing with the soil) and T3: (0% or crude land soil) (Table – 1; Fig–5). T1 and T3 were acted as +ve' and -ve' controls and T2 acted as a subject.

Table – 1: Treatment and their subject description of the experiment.

Treatment (T)	Subject	Matrix
Treatment-1 or T1	+ve' Control	Crude mine tailing
Treatment-2 or T2	Subject	Half mix tailing with soil
Treatment-3 or T3	-ve' Cntrol	Crude land soil

2.4. Preparation of seedlings for test pots and Plantation

The young plants of T. latifolia, S. spontaneous, P. vittata and C. compressus (Initial age – one month; plant length 30 cm approximately; 10 plants per treatment of individual species) seedlings were cut up to 15cm above the stem and 5cm below root portion. The seedlings were planted in the center of each plastic pot containing crude normal soil and tailing of study area by using a randomized design. After the plantation process was completed were placed into a controlled playhouse condition to assure constant temperature, humidity, and light. Plants were moisture manually once a day and harvesting was made at one-month intervals for four months for determining the growth parameters and metal accumulation.

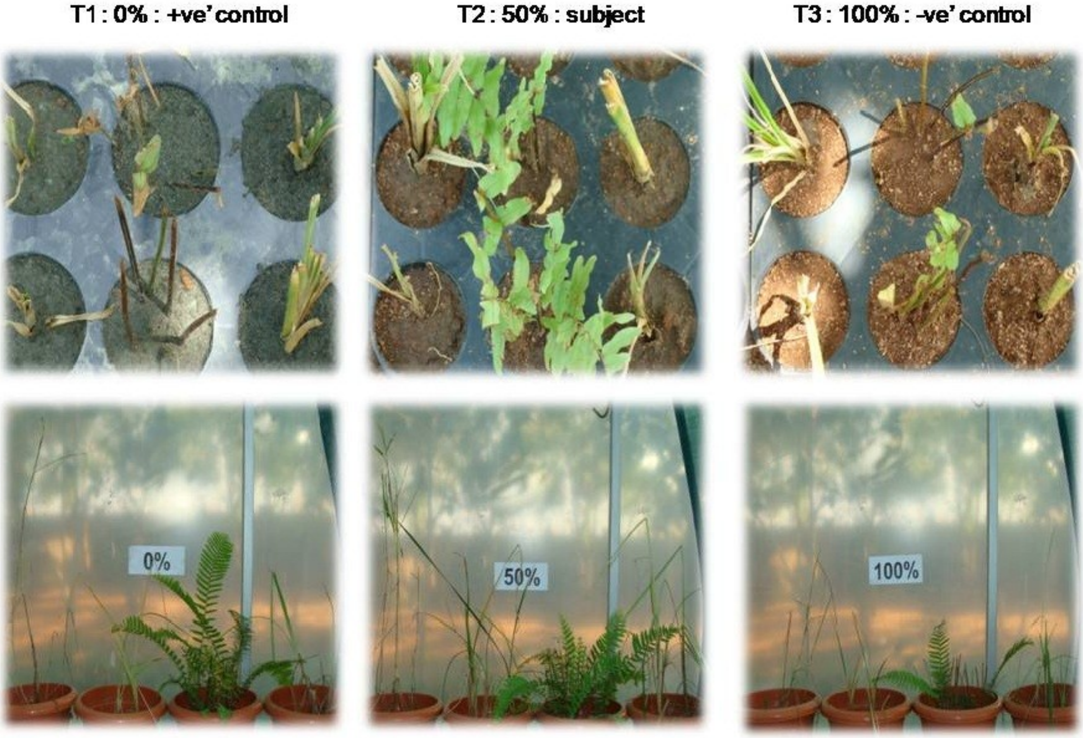


Figure – 5: Ex-situ Experimental setup: T1: +ve' control (100% or crude mine tailing), T2: subject (50% or half mix tailing with the soil) and T3: -ve' control (0% or crude land soil).

2.5. Growth performances of experimental plants

The pots were removed from the poly house after every one month interval. The plants and roots were removed from each pot. To understand amendment effect, the growth performances of plants were estimated in terms of height, fresh and dry weight, leaf number and survival rate. The number of plantlets survived every month was recorded for four months and survival percentage (SP) was calculated. Yield attributing parameters like average height, number of leaves, fresh and dry weight were recorded regularly. In summer they were watered to make up for the losses due to evapo-transpiration.

2.6. Sample collection

Before collecting the samples, containers were cleaned by soaking in 2 N HNO3, rinsed with pure water, and then air-dried in a fume hood. For determination of physico-chemical parameters, representative replicate random samples were sampled regularly in every 1-month interval by standard methods in each plant pot culture of respective treatment. For soil, samples were taken from the cultured pot of rhizosphere zone of plants. Cultured soil (~1kg) was taken from 15 cm depth in all the harvesting periods. Similarly, the plant samples were harvested from respective pot culture. Samples from crude land soil and tailing served as –ve' and +ve' controls respectively. The –ve' control serves as any negative effect of the added amendment on plant growth and metal uptake. [40][41][36][42]. Collected samples from the pot culture during 0-time and every one month interval of 1st, 2nd, 3rd and 4th months of phyto-remediation process, the data (D) were recorded as D0, D1, D2, D3 and D4 respectively.

2.7. Sample preparation

For sample preparation, reagents and water used here were of analytical grade. The pot soil samples were crushed, mixed thoroughly and air-dried for 5 to 6 days, then dried in hot air oven for 24 hrs at 65^{o}C and finally ground into fine powder to pass through a 2 mm sieve [42]. Plant: The collected plant samples were cleaned with tap water and were again cleaned with distilled water. Individual plant species were air-dried and weighed (fresh weight) and kept at 110º C for 2 days in hot air oven and dry weight were taken. After drying process the samples were ground into fine powder with a coffee grinder (Kenstar mixer grinder MG 0411) to pass through a 2 mm sieve [43][44].

2.8. Instrumentation and Analytical Procedure

After appropriate preparation, replicate samples were analyzed separately by following standard protocol. The results were calculated on a dry weight basis. Physico-chemical analysis of pot soil (row and subject) samples was carried out for all the parameters from D0, D1, D2, D3 and D4 months of Phyto-remediation process. The pH (solid: deionised water = 1:2.5 w/v) [45] and electrical conductivity-EC (solid: deionised water = 1:2 w/v) [45] of the samples were measured by using the electro pH meter and the electro conductivity meter by allowing to equilibrate for 30 minutes. For metal analysis, a known quantity (0.05g) of prepared soil sample was digested with 10 ml of acid mixture (7:3:1 concentrated HF-HNO_3-$HClO_4$) in lid covered PTFE Teflon beaker heated at 200°C. After the one hour completion of the digestion, the lids were then removed and the contents were evaporated to incipient dryness until a crystalline paste was obtained. The remaining residues were then dissolved using 10 ml of 1:1 HNO3: H2O and kept on a hot plate for 10 minutes at 70°C to dissolve all suspended particles. 1 ml (5μg/ml) of Rhodium (Rh) solution was added to act as an internal standard and then the volume was made up to 250 ml with purified water (18 MΩ) stored in polyethylene bottles for the determination of heavy metals [40]. Plant: harvested plant sample of 0.5g dried tissues mixture were digested in a Teflon container by adding HNO_3 (65%) and 1ml of 30% of H_2O_2. After microwave/hot plate digestion cycle, digested samples were made up to 25 ml with the de-ionized water [46]. Radionuclide's and metals (Uranium-U, Manganese-Mn, Iron-Fe, Vanadium-V, Nickel-Ni, Copper-Cu and Zinc-Zn) in soil and plant samples were analysed by Inductively Coupled Plasma Mass Spectrometry (ICPMS make PerkinElmer Sciex ELAN DRC II)) at the Central Research Facility available at National Geophysical Research Institute (NGRI), Hyderabad. The international geo-standard certified values of SO-1 for soil [47], and NIST for plant sample were used for standard references. Subsequently, results were corrected using blanks.

2.9. Determination of Transfer Factor (TF)

Soil-to-plant transfer factor is one of the important parameters used to estimate the concentrations of radionuclides in plants according to a transfer model. The uptake of radionuclides or elements by plants from the soil is normally described as transfer factor (TF), i.e. the ratio of concentration of radionuclides or an element in plant tissue and soil (in Bq.kg-1 or mg.kg-1) [48][49][50][51] and it is represented as below.

$$TF = \frac{\text{Metal concentration in Plant tissue (Dry weight)}}{\text{Metal concentration in Soil (Dry weight) from where the plant was grown.}}$$

The transfer factors vary significantly according to plant properties and soil type [50].

III. Results And Discussion

T. latifolia, S. spantanium, P. vittata and C. compressus plants were selected for ex-situ experiments. Each selected plant species have been exposed for the same treatments/concentration levels (T1, T2 and T3). Growth performance and amount of metal uptake by these plants in all the treatments at every 1 month interval period (total 4 months) and the transfer factor from soil to plants are described here under (detailed in annexure 7.1 and 7.2). The analysis indicated that the concentrations in soils and plants for all study treatment differs significantly with respect to the contents of the examined elements.

3.1. Initial plant, soil and tailing analysis:

The tailing was shown much higher concentration levels of seven elements – Aluminum , Manganese, Iron, Nickel, Copper, Zinc and Uranium. The concentrations of other metals are significantly in low concentration [39]. Initial data (D0 or 0-time) of plant seedlings and crude soil indicated that V, Mn, Fe, Zn, Ni, Cu and U were present in various concentration levels, but were within national 503.13 standards [52]. However, the concentrations of U in the plant species were within the ranges of the average values given for all types of plants [43]. The pH, EC and metal data were sufficiently within control permissible limits or threshold values (Table – 2).

Concentration of toxic metals were observed high in the raw mine tailing in the order of Fe: 43180 > Mn: 690 > Cu: 320 > Ni: 220 > V: 150 > U: 40 > Zn: 30 mg kg^{-1} dw, respectively and significant changes were observed after treatment

Table – 2: The Chemical properties of initial (D0) tailing, soil, plant seedling and control used in the study.

0 - Time (or) of D0= sample analysis data (metals in ppm & EC in mmohs/cm)									
Plant seedlings									
Plant sp.	V	Mn	Fe	Ni	Cu	Zn	U		
T. latifolia	0.08	3.21	20.67	0.08	0.21	0.47	0.04		
S. spontanium	0.58	3.86	101.58	0.09	0.62	1.21	0.05		
P. vittata	0.10	0.33	17.98	0.01	0.14	0.22	0.01		
C. compressus	0.20	6.94	83.57	0.09	0.34	0.74	0.08		
Soil/tailing									
Treatment	V	Mn	Fe	Ni	Cu	Zn	U	pH	EC
T1 (raw tailing)	150	690	43180	220	320	30	40	7.6	2.67
T2 (50%-tailing)	90	600	32210	140	160	40	33	7.2	12.5
T3 (raw Soil)	40	450	23800	40	10	50	27	5.9	80.9
Control soil									
Control	10	510	6414	89	69	92	4	6.1	0.3
Control soil samples collected from Rankini mandir/ Rainibeda/ Bhatin/ Chatikocha/ and Thilaitand villages resides around the tailing ponds									

3.2. Growth performances of experimental plants:

The growth parameters studied under pot culture experiments are described below (detailed in Annexure – 7.1). All plants were intended to grow for four months in three different soils. Since the pots used for the experiment contained less than 5 Kg of soil, complete growth of these plants as in fields was not expected. The plants grown luxuriantly on subject soil and raw soil throughout the experiment and results of their height, weight, leaf numbers and survival percentage were depicted in (Fig–6 & Fig–7).

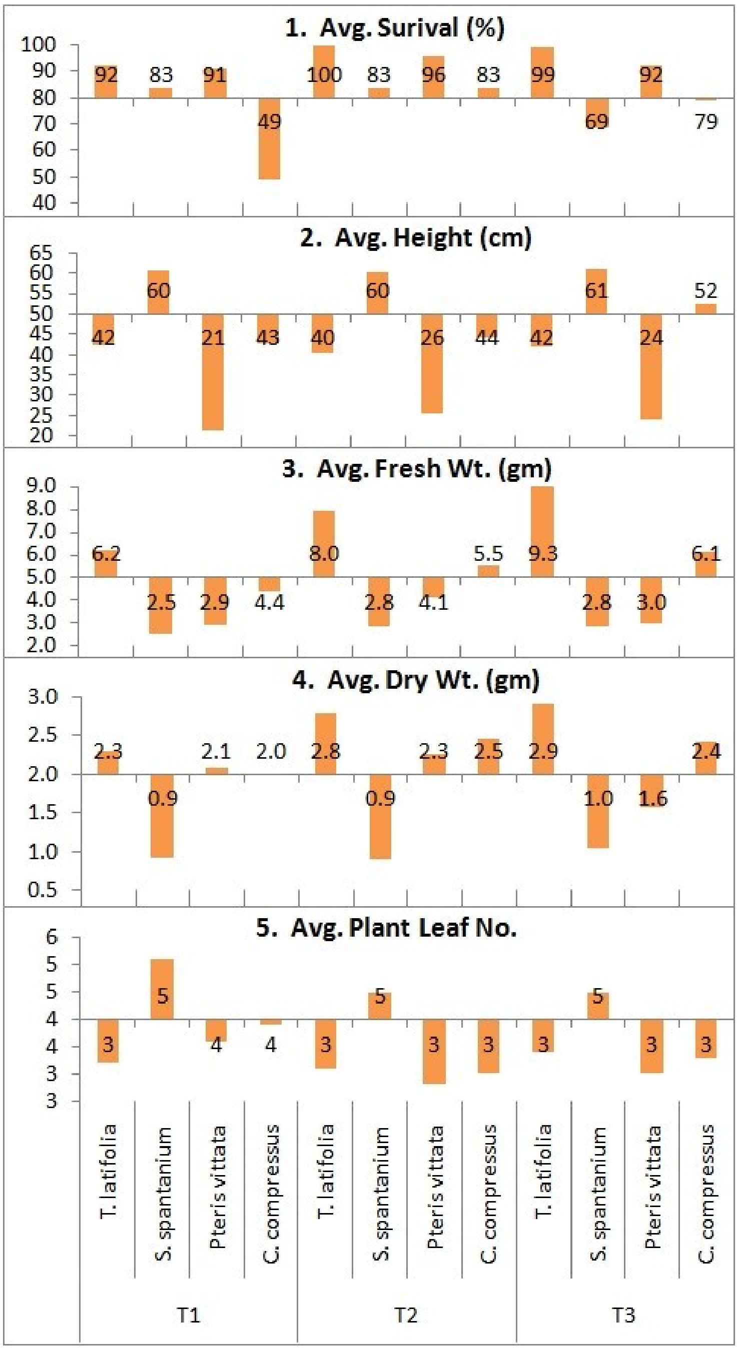
3.2.1. Survival (%): The results of the survival percentage of plants provide the useful information concerning to growth responses of the plants. The number of plants survived every month was recorded for every months and survival percentage was calculated. The highest survival rate was observed in T2 > T3 > T1. The plants were killed in T1 may due to high metal contamination in the soil. After four month of plantation, the maximum achieved survival was recorded as 91% > 85% > 79% in T2 > T3 > T1 and P. vittata > T. latifolia > C. compressus > S. spontanium respectively.

3.2.2. Plant height: The plant height of all the individual plants in a pot was measured with measuring staff or scale in cm. The highest plant height was observed in T3 > T2 >T1. The results of the height of plants showed that it was varied from plant to plant after 1 to 2 months of Phyto-remediation. After 2 month of Phyto-remediation, all the plants showed the proper growth as evidenced by vigorous height of plants. The highest growths in the height of plants were observed in the order 44.77, 42.60, 41.62, cm in the plants T3, T2, T1 after end of the experiment.

3.2.3. Number of leaves: Leaf number of the plants was dependent on plantation periods and it varied from plant to plant. Number of leaves was calculated by actual counting of leaves per branch. At the end of each harvest, numbers of leaves counted / plant (only full leaf, and not all the parts emerging out of main stem). The highest plant leaves was observed in T1 > T3 > T2. The maximum leaf number was observed in T2 and T3 after 3 month and in T1 is after 4 month of experiment i.e. an increasing trend was observed with increase in Phyto-remediation period.

3.2.4. Fresh and Dry weight: The collected plant samples were cleaned with tap water to remove any particles bound to root or shoot portion. Individual plant species were air-dried and weighed (fresh weight) and kept at 110º C for 2 days in hot air oven and dry weight were taken [43]. The highest plant fresh weight was observed in T3 > T2 > T1, and the highest plant dry weight was observed in T2 > T3 > T1. It was noticed that the weight gained by plants from T1 is less than that from T3 or T2 soil. It may be due to the variation in physical and chemical characteristics of the each pot soils.

Figure – 6: Growth parameters study of the experimental plants at different treatment.



3.2.5. Plant health of during experiment: A considerable increase in growth occurred in soil amended tailing. The addition of soil increased the growth levels (Fig – 3). The growth performances of plants in reference to height, fresh weight and leaf number were found to increase with increase in Phyto-remediation periods particularly after second month. The increase in growth parameters was showed by all the plants but varied from species to species. Overall results indicated that the plant P. vittata showed the highest growth performance followed by T. latifolia. It is also observed that the addition of soil was found significant change in pH to alleviate the toxic effects that heavy metal have on plant health, hence the enhanced growth parameters such as survivability, height, fresh and dry weigh of plants was reached. In terms of number of leaves assessed, the treatment did not show significant beneficial effect possibly due to the rather short experimental period of 4 months. In this scenario, addition of water application plays a major role on the plant health i.e. for the survival and growth of the plants. Continuous moisturising has been seen the positive effect on the growth of P. vittata and same may lead the negative effect on S. spontanium. Hence, vigorous growth has been achieved in P. vittata even after completion of experiment and least survival was seen in S. spontanium.

3.3. Soil pH and EC and their affect on expriment

The results of pH, EC and chemical properties of raw and phytoremediated pot culture showed significant changes during experiment in the growth of various plants (Fig–8). The pH of T1 (7.6) at initial was found to be higher therefore they can be defined as moderately saline tailing followed by T2 (7.2) and T3 (5.9) while after Phyto-remediation it was increased from 7.6 to 7.8; 7.6 to 7.7 and 6.9 to 7.3 respectively after 4 months of remediation. The observed increased in pH with increase in treatment period was attributed to the 'alkalizing' effect of soils. The EC at initial was found to be low in T1 (2.67) followed by T2 (12.5) and T3 (80.9) and drastically increased with increase in treatment period. After experiment the optimum pH of T1, T2 and T3 were 7.7, 7.6 and 7.2 respectively and for EC 225.4, 86.7 and 62.3 mmohs/cm respectively. With compared to treated T1, soil addition was lowered the pH and EC (i.e. up to 7.6 and 86.7 mmohs/cm respectively). But the same soil addition, when compared with initial T1 it was shown that no significant changes in pH or decreased in EC were reached. However, in either way with or without amendment, the same experiment with the plantation enhances the pH and EC at great level, therefore the work directed that along with amendment it is to be invading the plant species to consolidate the contaminants in mine waste. Therefore due to several changes that occur with lowering pH and EC, the increased growth of the plants was observed in treatment T2 than T1.

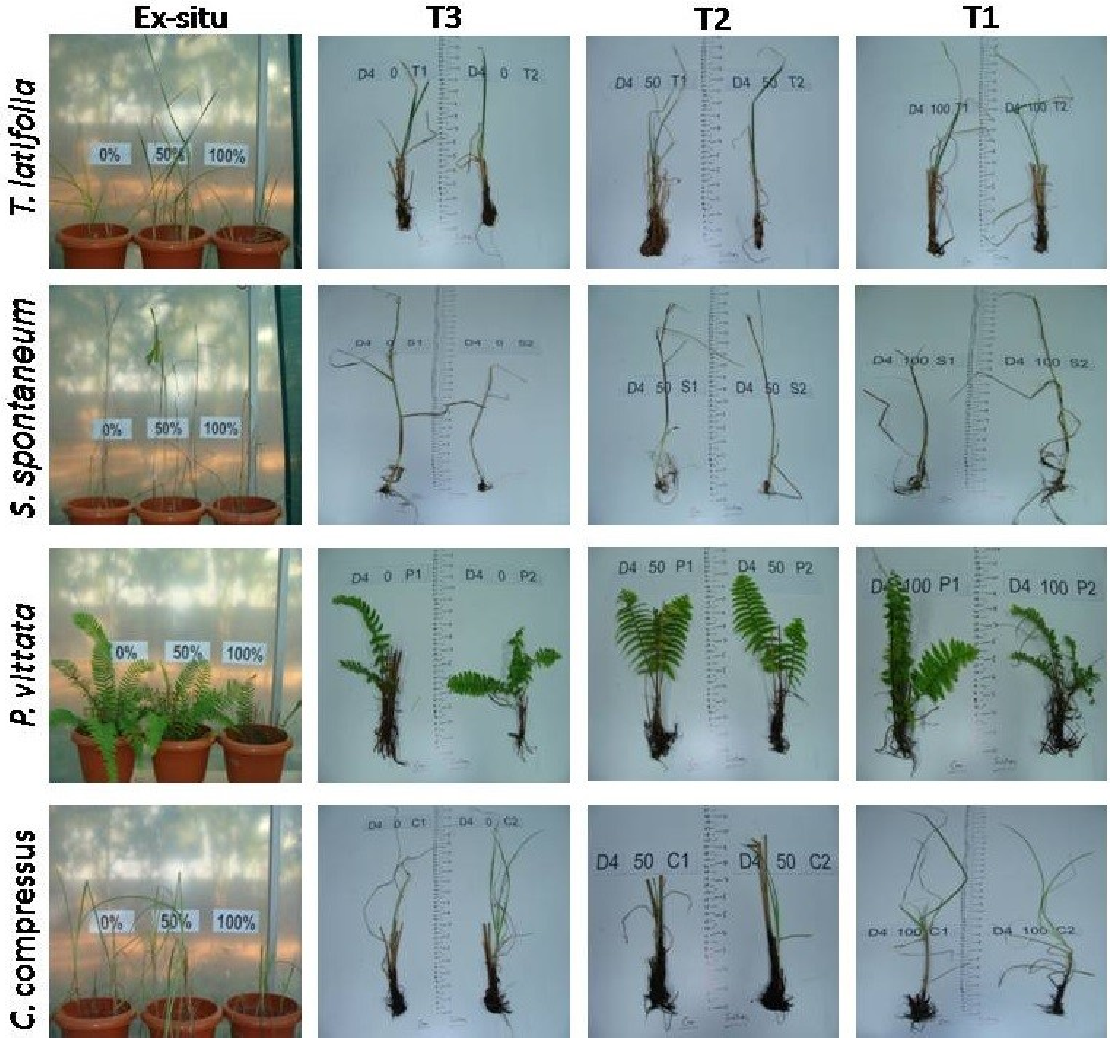


Figure – 7: Plant health of the selected spp. at different treatment during experiment.

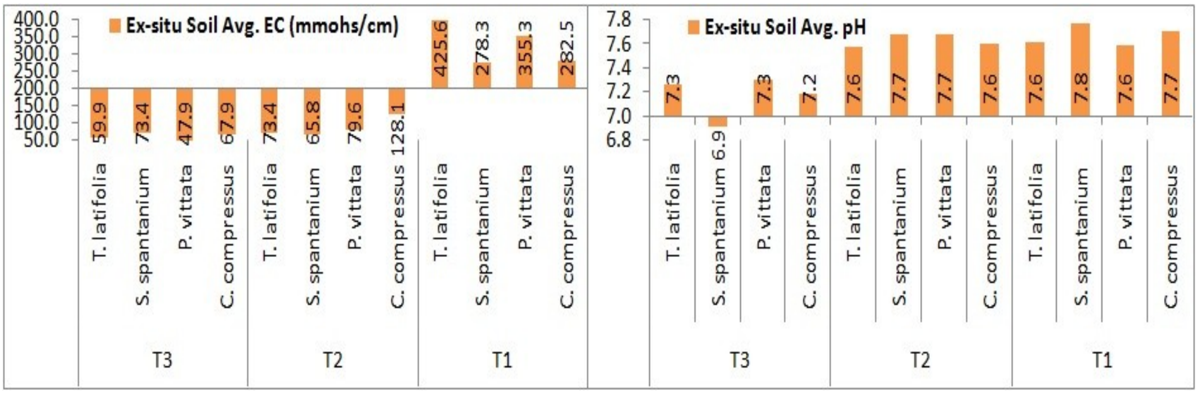


Figure – 8: Average values of pH and EC in soil samples collected from different treatment.

3.4. Metal analysis of soil and plant samples:

3.4.1. Soil metals characteristics

Except in some elements in some circumstances, there was no or minute increasing and decreasing change of elemental concentration was achieved during and after Phyto-remediation. The experiment cleared that without addition of land soil in tailing was also shows the enhanced Phyto-remediation or decreased concentration of contaminants in treatment T1. However, the average metal concentration in tailing or treatment T1 was consolidated with addition of land soil i.e. in T2 at great level (Fig–9).

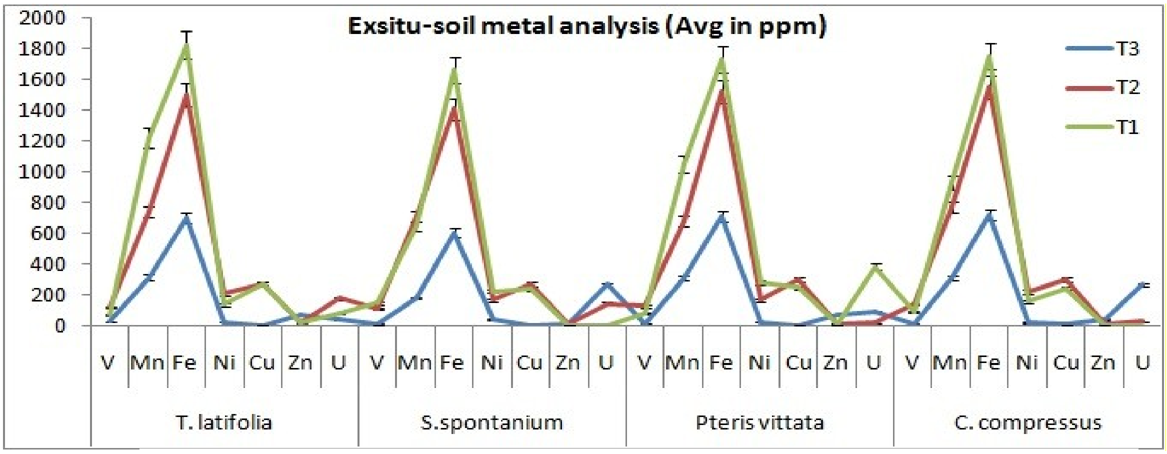


Figure – 9: Soil metal characteristics in three different treatments - T1, T2 & T3 during and after Phyto-remediation

3.4.2. Plant metals characteristics

The plants growing in experiment had been bio-concentrated substantial amount of toxic metals in the order Fe > Mn > Ni > Cu > Zn > U > V. The metal concentration was initially low which increased with the growth of the plant as evidenced by increased metal accumulation in the plant tissues (Fig–10). Except Uranium, all metals shows similar pattern of accumulation from soil to plants. The maximum bio-concentration of Uranium was seen in S. spontanium and C. compressus from treatment T1 pot soil and least concentration was seen in P. vittala. But the P. vittala followed by C. compressus show the maximum bio-concentration of Uranium from treatment T2 pot soil. This due to the tailing structure and nutrient supplies were elevated by the land soil additions. Soil also provided an increase in porosity, water holding capabilities, and aeration of the tailing [53][54].

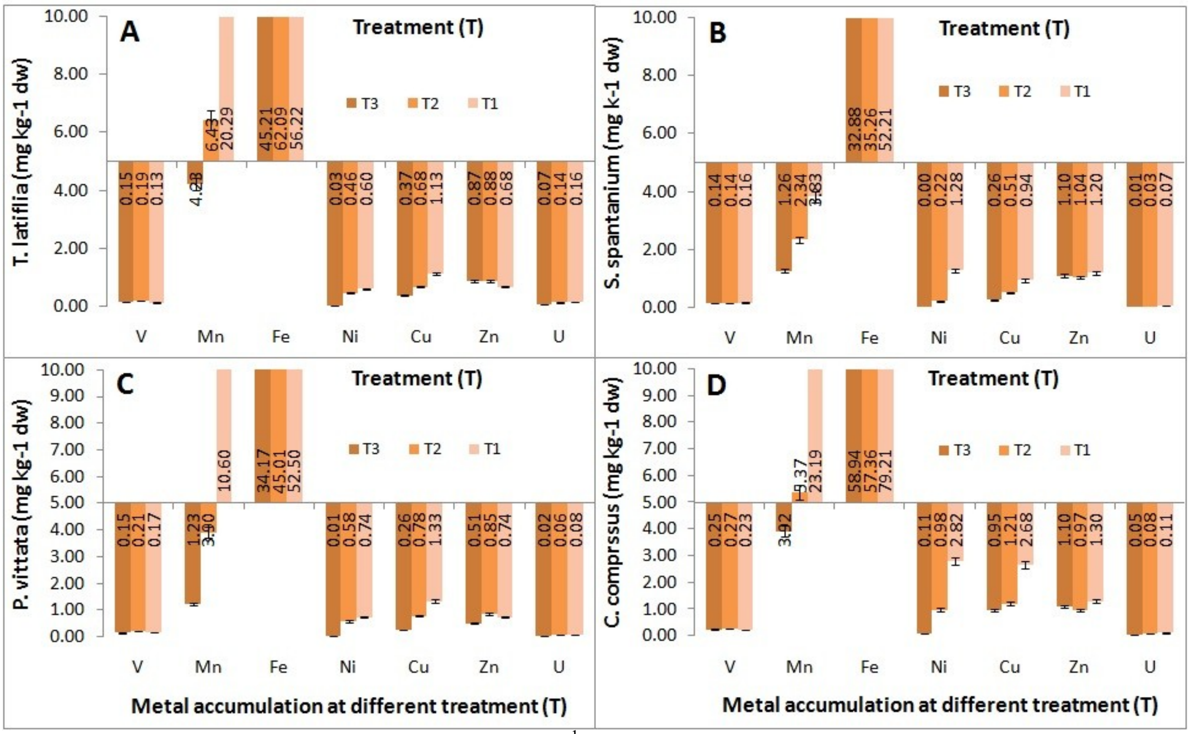


Figure – 10: A-D: Metal bio-concentration (mg kg–^{1} dw) of selected plants during the Phyto-remediation of Uranium mine tailing at EPTRI, Hyderabad (A.P.). All the values are mean of replicate samples and bars represents ± SE (n = 5). (T3 – Treatment 3; T2 – Treatment 2; T1 – Treatment 1; dw – dry weight).

The highest V, Mn, Fe, Ni, Cu and Zn (0.25, 10.83, 65.17, 1.30, 1.61 and 1.12 mg kg^{-1} dw respectively) bio-concentration was found in the plant C. compressus and lowest V, Mn, Fe, Cu and U concentration recorded in the plant S. spantanium. In the case of U, the highest bio-concentration potential was showed by the plant T.

latifolia (0.12 mg kg^{-1} dw). The lowest concentration of Ni and Zn were seen in T. latifolia and P. vittata respectively. The plant T. latifolia indicated similar concentration capabilities for the V, Mn, Fe, Ni, Cu and U. T. latifolia and P. vittata were represented the similar higher V, Mn, Ni, Cu and U accumulation capabilities as compared to other species. C. compressus shows similar accumulation of Cu and Zn. After four months of phyto-remediation, results of metal concentration capabilities of selected plants focused that the plant C. compressus have the highest potential to concentrate V, Mn, Fe, Ni, Cu and Zn; while the plant T. latifolia was showed the highest accumulation capabilities of U. Other plants were also found effective in bio-concentration of above metals but these concentrations were defers as comparatively. Maximum accumulation of metal were fund at first month of harvested samples in T. latifolia, C. compressus and P. vittata while in S. spantanium the maximum accumulation was at fourth month of harvested samples. However, irrespective of time period, less or more the accumulation will taken place every month and even after.

The findings of present study indicated that bio-concentration capabilities differ plant to plant and pant to element. Some elements in some plants enhanced accumulation and the same elements in other plants decrease accumulation was seen with increase in maturity period, that increase or decrease in metal accumulation by plant tissues of different maturity may also be due to increased or decreased permeability and metabolic activities associated with increasing age. The observed elevated levels of heavy metals in the soils are reflected by the high content of Mn and Fe in the harvested samples of T. latifolia, C. cmpressus and P. vittata plants and only Fe in S. spantanium within all the treatments and harvesting period. The relatively low uptake of other metals by all the species examined may also be attributed to the increased pH of the soils.

3.5. Transfer Factor (TF)

The accumulation ratio is an important factor in understanding the relative availability of trace elements to plants [55][56]. The results of transfer factor for metals with respect to individual plant species was presented in Fig–11 (details in Annexure – 7.2). The plant samples from different treatments have shown unique characteristic nature for each metal in their TF and not any plant have the same characteristic metal TF in the same treatment or in different treatment. The highest transfer factor was found for Zn followed by Cu and Mn in all the plants but plant S. spantanium also showed a high transfer factor (0.01) for the U. Data in general showed that the plants concentrated a high amount of metals, vis a vis their uptake differ from one species to other. Such a high metal transfer factor shown by these plants resulted into lowering the metal content of tailing and improvement in physico-chemical characteristics of the treated tailing. The TF of U in the plant species were within the ranges of the average values given for all types of plants [43]. The harvesting of plant in every month interval was found that U and Mn uptake in all plants at is more apparent at 1^{st}- month (D1) interval. Remaining all other metal has no significant variation in TF in experimental plant species when comparing between months. Sequences of accumulation ratios established for plants indicated the following heavy metal absorption capability of T. latifolia: Zn > Cu > Mn > V > Ni > Fe > U, S. spantanium: Zn > Cu > Mn > U > V > Ni > Fe, P. vittata: Zn > Cu > Mn > V > Ni > Fe > U and C. compressu: Zn > Cu > Mn > Ni > V > Fe > U. The Zn > Cu > Mn accumulation sequences were similar for each of the examined species. The high accumulation ratio values for Zn were characteristic for all the investigated species indicate high accumulation ability.

3.5.1. Typha latifolia: Addition of soil amendment clearly affects the metal TF in T. latifolia and it had significantly negative effect of V, Mn, Ni, Cu and U and the positive accumulation has seen only in Fe and Zn. Except Zn (which shows maximum at third month stage), The maximum TF had seen at first month stage and least at fourth month interval for V, second month interval for Zn and it was at third month harvested samples for Mn, Fe, Ni, Cu and U, hence these species showed the decline accumulation in after one month Phyto-remediation. In average, this plant shows the maximum TF for Zn (0.027) followed by Cu (0.026) and Mn (0.013) and the least TF was seen for U (0.001). The best suitable trartment and time of harvesting of T. latifolia for phyto remediation or accumulation of above study individual contaminants are as follows: V: D1-T1; Mn: D1-T1; Fe: D1-T2; Ni: D1-T1; Cu: D1-T1; Zn: D3-T2; U: D1-T1 (Fig–11-A).

3.5.2. Saccharum spontaneum: Following soil amendment, as seen in T. latifolia, this plant also had significant decreasing or negative effect on TF for all the contaminants. V, Mn, Cu and Zn were shows the maximum TF at second month harvested samples and for Ni and U the same was seen at third month samples. The least TF for V, Mn and Cu was at third month stage; hence these species showed the decline accumulation in after two month of Phyto-remediation and the same was seen for Ni and Zn at fourth month and for U at second month. However, addition of amendment, there is no variation in TF has been seen for Fe at any stage of harvest. In average, this plant shows the maximum TF for Zn (0.09) followed by Cu (0.035) and Mn (0.005) and the least TF was seen for Fe (0.001). The best suitable trartment and time of harvesting of S. spontanium for phyto remediation or accumulation of above study individual contaminants are as follows: for V: D2-T2; Mn: D2-T1; Ni: D3-T1; Cu: D2-T1; Zn: D2-T1; U: D3-T1 (Fig–11-B).

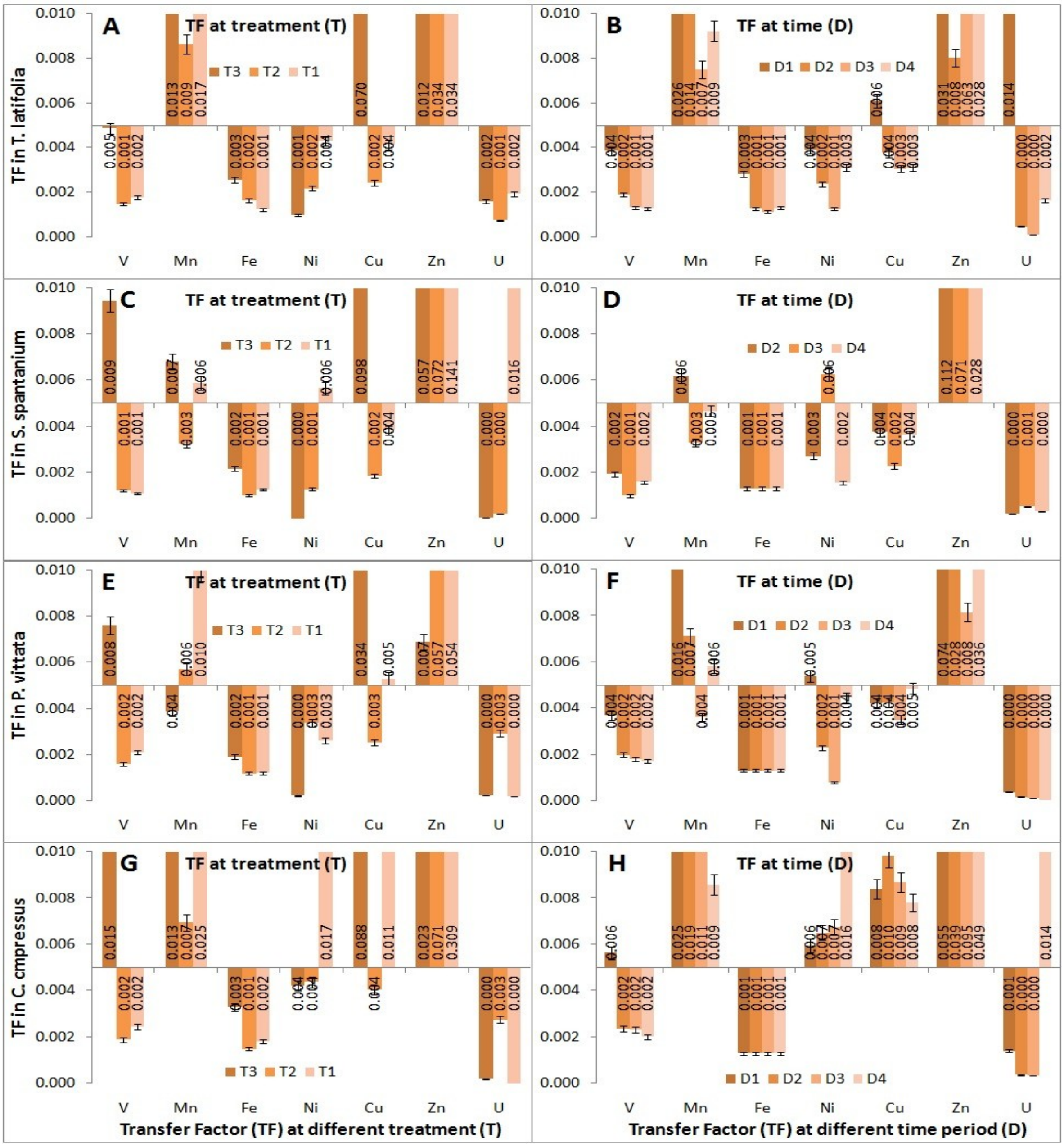


Figure – 11: A-H: Transfer Factor capabilities of selected plants during the Phyto-remediation of Uranium mine tailing at EPTRI, Hyderabad (A.P.). All the values are mean of three treatment and bars represents □± SE (n = 5). D1 - 1st – month data; D2 - 2nd – month data; D3 - 3rd – month data; D4 - 4th – month data; dw – dry weight; T1 – Treatment 1; T2 – Treatment 2; T3 – Treatment 3.

3.5.3. Pteris vittata: Except in Ni, Zn and U, addition of soil amendment clearly promotes the negative effect on TF for all the contaminants. Except Cu which shows the maximum TF at 4th month stage, all the other contaminants shows the same after one month harvested samples. In context of least accumulation, for V and U at fourth month and all other elements the least accumulation was shows after at third month stage harvested samples. However, addition of amendment, there is no variation in TF has been seen for Fe at any stage of harvest. In average, this plant shows the maximum TF for Zn (0.0039) followed by Cu (0.014) and Mn (0.007) and the least TF was seen for U (0.001). The best suitable trartment and time of harvesting of P. vittata for phyto remediation or accumulation of above study individual contaminants are as follows: V: D1-T1; Mn: D1-T1; Ni: D1-T2; Cu: D4-T1; Zn: D1-T2; U: D1-T1 (Fig–11-C).

3.5.4. Cyperus compressus: Except U which shows increased accumulation or positive effect on TF, addition of soil amendment clearly affects the metal accumulation in C. compressus plants and it had significant decreasing accumulation or negative effect on TF. Unlike other plants, Cyperus compressus has specific accumulation period for each elements. The maximum accumulation of V and Mn were seen in after one month harvested samples and for Ni and U the same was seen at after forth month samples. For Cu and Zn, the maximum accumulation was seen in after second and third month samples respectively. In context of least accumulation, this plant shows the least accumulation of Ni, Zn and U after at first, second and third month and for V, Mn and Cu the same was seen after at fourth month harvested samples respectively. However, addition of amendment, there is no variation in TF has been seen for Fe at any stage of harvest. The best suitable trartment and time of harvesting of C. compressus for phyto remediation or accumulation of above study individual contaminants are as follows: V: D1-T2; Mn: D1-T1; Ni: D4-T1; Cu: D2-T1; Zn: D3-T1 and U: D4-T2 (Fig–11-D).

Data reveals that, irrespective of harvesting time interval, the addition of soil amendment clearly affects the metal accumulation and it had significant decreasing TF of contaminant accumulation in T. latifolia, S. spantanium, P. vittata and C. compressus. This effect due to non bioavailability of contaminants to the pant and an increase of pH and organic content resulted with the addition of land soil to crushed rock residues of mine tailing. The addition of soil was found to alleviate the toxic effects that heavy metal have on plant health, hence therefore the enhanced growth parameters such as survivability, height, fresh and dry weigh of plants was reached.

3.6. Identification of plant species for multi accumulation and non-accumulation of contaminants

The plant species having their own bio-concentration and also can accumulate the different elements at different concentration level. The pants can bio-concentrate few elements and the same plant may not be a accumulator of the same elements. Therefore, in this study in order to identify elite plant species for remediation and consolidation of tailing pond, hyper and non-concentrator or accumulators of plant species need to be sorted based on their elemental contaminants (Table-3). By seeing simultaneous accumulations of multiple elements, only C. compressus has shown highest multiple contaminants concentration and accumulation (i.e. up to all the above elements – AL, Mn, Fe, Ni, Cu, Zn and U) followed by moderate Bio-Concentration in T. latifolia (i.e. up to three elements – Mn, Fe and U) and moderate accumulation in P. vittata (i.e. up to four elements – V, Ni, Cu and U). While seeing in plant species with simultaneous non-accumulation of multiple elements, only S. spantanium has shown least multi contaminants bio-concentration and accumulation (i.e. it can accumulate only up to one element – Zn) followed by moderate Bio-Concentration in P. vittata (i.e. up to three elements – V, Ni and Cu) and moderate accumulation in T. latifolia (i.e. up to two elements – Mn and Fe). A recent study reported that high transfer factors for Cyperus spp. suggested the high potential of this species over other plant species for metal accumulation [43]. The highest and lowest multi-metal accumulation patterns characteristic of C. compressus and S. spantanium respectively has been confirmed in this study.

Table – 3: Plant species with simultaneous multi elemental accumulation and non-accumulation of contaminants.

Experiment	Parameter	Grade	Suggested Plants	Multi Contaminants						
				V	Mn	Fe	Ni	Cu	Zn	U
Bio-Concentration	Concentration	Hyper	C. compressus	V	Mn	Fe	Ni	Cu	Zn	U
		Moderate	T. latifolia	-	Mn	Fe	-	-	-	U
	Non-Concentration		P. vittata	V	-	-	Ni	Cu	-	-
		Non	S. spantanium	-	-	-	-	-	Zn	-
Accumulator (TF)	Accumulator	Hyper	C. compressus	V	Mn	Fe	Ni	Cu	Zn	U
		Moderate	P. vittata	V	-	-	Ni	Cu	-	U
	Non-Accumulator		T. latifolia	-	Mn	Fe	-	-	-	-
		Non	S. spantanium	-	-	-	-	-	Zn	-

3.7. Plant species recommended for remediation of Uranium tailing ponds

Elsevier reported that the samples with high V, Fe, Ni, Cu and Zn concentration may due to geochemical origin, aquatic process such as neutralization, precipitation, flocculation as well as adsorption occurred in the receiving water during manning to milling and acid leaching [57][58]. And the samples with high Mn and U concentration may due to technical limitations that all of the uranium present in the ore cannot be extracted. Therefore, the sludge also contains 5% to 10% of the uranium initially present in the ore, hence the index of the contamination related to uranium mine is uranium [59][35][58]. The samples with high Mn concentration may due addition of manganese dioxide or $KMnO_4$ used as oxidant in acid leaching uranium circuit and also common contaminant in mining process [60][61][38][58]. Therefore, of the above seven elements, only two elements: U and Mn were identified as major contaminants in the selected sites that need to be remedied. Apart from this, analysis results of metals and radionuclides in ex-situ experimental studies, different plant species have shown different bio-concentration and accumulation patterns of different identified contaminants. Therefore, keeping in view that the Phyto-remediation (accumulation and non-accumulation or consolidation) of contaminants – V, Fe, Ni, Cu and Zn) along with major contaminants – Mn and U, C. compressus and S. spontanium plant species have been selected for accumulation and non-accumulation or consolidate respective contaminants in this study. For maximum accumulation of U, soil amendment was best suited for C. compressus and the harvesting need to be done at after fourth month of plantation. For non-accumulation or consolidation of tailing, soil amendment was best suited and S. spontanium plantation was greatly recommended.

3.8. Quality Assurance:

The reproducibility of these procedures was compared to the results of an inter laboratory study by digesting and analyzing the reference material (Lucid Laboratories Private Limited, Hyderabad, India) for quality assurance using the more sensitive technique of ICP-OES Varian Liberty and the results are presented in Fig–12 shows a comparison of the results. Values were found to be within 97±4%. It is also compared with the previous works of the study area and all the results presented here are more or less following to the published works.

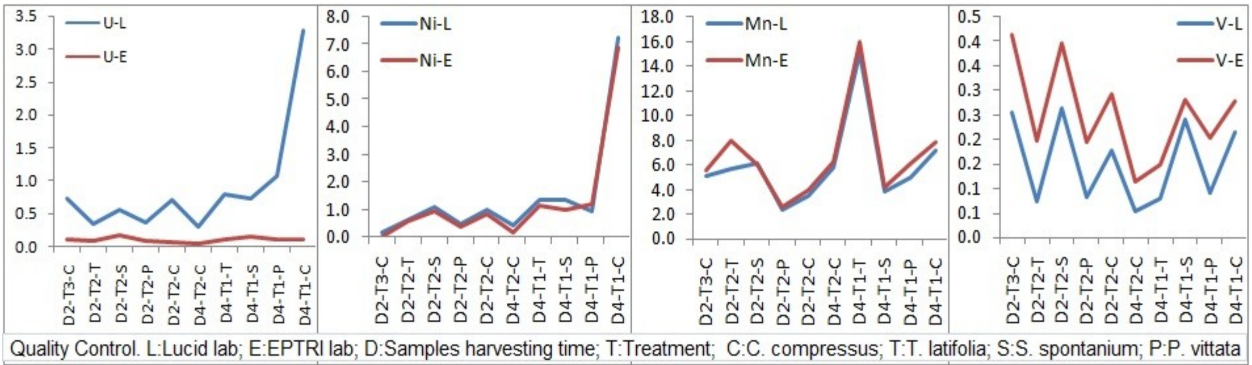


Figure – 12: Quality checking of inter- laboratory data (L- Lucid Laboratories Private Limited; E- Environment Protection Training & Research Institute)

IV. Conclusion

Ex-situ Phyto-remediation study was performed to identify the native plant species with hyper and non-accumulation or consolidation of contaminants in Uranium mine tailing ponds by amending land-soil. The addition of soil was found to alleviate the toxic effects that heavy metal have on plant health, hence therefore the enhanced growth parameters such as survivability, height, fresh and dry weight of plants was reached. Data reveals that, irrespective of harvesting time interval, the addition of soil amendment had significant decreasing TF of contaminant accumulation in *T. latifolia*, *S. spantanium*, *P. vittata* and *C. compressus.* This effect due to non bioavailability of contaminants to the pant and an increase of pH and organic content resulted with the addition of land-soil to crushed rock residues of mine tailing. For simultaneous bio-concentration and accumulation of multiple elements, only *C. compressus* have shown highest multi-contaminants (i.e. up to all the above elements – AL, Mn, Fe, Ni, Cu, Zn and U) capability of the same and the harvesting need to be done at after the fourth month of the plantation. While seeing in plant species with simultaneous non-accumulation or consolidation of the tailing of multiple elements, only *S. spantanium* has shown least multi-contaminants bio-concentration and accumulation (i.e. it can accumulate only up to one element – Zn). In both the cases, soil amendment was best suited and *S. spontanium* plantation was greatly recommended. Apart from all, addition of land-soil in radionuclide and heavy metal contaminated soil will gives a positive effect on nutrition and growth of a vegetation cover which in turn improve the stability and sustainability of the remediated site with less risk of metal dispersion which is threatening to living things.

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VI. ANNEXURE

Annexure – 7.1: Table with growth parameters of selected plant spp. At different treatment and sampling time (Matrix: Pant).

Growth parameters					
Total Avg	**Fresh Wt. Avg. (Gm)**	**Dry Wt. Avg. (Gm)**	**Avg. No. of Leafs**	**Survaivability(No's)**	**Height(cm)**

Plant	Trtmt	D0	D1	D2	D3	D4	Avg	D0	D1	D2	D3	D4	Avg	D0	D1	D2	D3	D4	Avg	D0	D1	D2	D3	D4	Avg	D0	D1	D2	D3	D4	Avg
T. latifolia	T1	0	9	8	4	10	6	0	3	3	2	4	2	0	2	5	4	5	3	18	17	16	16	16	17	15	40	41	60	56	42
S. spantanium		0	2	4	4	3	2	0	1	1	2	1	1	0	3	7	10	6	5	18	18	14	15	10	15	26	52	78	93	55	60
P. vittata		0	4	3	3	4	3	0	3	2	2	3	2	0	2	5	5	6	4	18	10	18	18	18	16	20	21	18	23	26	21
C. compressus		0	5	8	5	4	4	0	2	3	3	2	2	0	2	3	7	8	4	18	6	6	7	7	9	19	20	37	56	82	43
T. latifolia	T2	0	9	13	9	9	8	0	4	3	3	3	3	0	2	4	5	5	3	18	18	18	18	18	18	15	35	56	50	47	40
S. spantanium		0	3	6	4	2	3	0	1	2	1	1	1	0	3	7	9	4	5	18	16	14	14	13	15	26	58	90	78	51	60
P. vittata		0	6	5	5	5	4	0	4	2	3	2	2	0	2	4	4	4	3	18	14	18	18	18	17	20	22	28	28	31	26
C. compressus		0	10	8	6	5	6	0	4	3	3	2	2	0	2	4	5	4	3	18	15	15	16	11	15	19	49	67	62	24	44
T. latifolia	T3	0	14	12	10	11	9	0	3	4	3	4	3	0	2	5	6	4	3	18	18	18	18	17	18	15	38	60	61	36	42
S. spantanium		0	2	5	3	4	3	0	1	2	1	1	1	0	3	6	8	6	5	18	17	9	12	6	12	26	61	96	55	70	61
P. vittata		0	6	3	3	3	3	0	3	2	1	2	2	0	2	4	4	5	3	18	12	18	18	17	17	20	22	22	27	30	24
C. compressus		0	8	8	7	7	6	0	3	3	3	3	2	0	2	4	6	5	3	18	10	15	15	13	14	19	38	64	83	59	52

Annexure – 7.2: Table with elemental concentrations and transfer factor at different sampling time and treatment (Matrix: Soil and Pant).

Elemental Concentration																							
Plant	**Treatment**	**Time**	**V**			**Mn**			**Fe**			**Ni**			**Cu**			**Zn**			**U**		
			P	**S**	**TF**	**P**	**S**	**TF**	**P**	**S**	**TF**	**P**	**S**	**TF**	**P**	**S**	**TF**	**P**	**S**	**TF**	**P**	**S**	**TF**
T. latifolia	**T3**	D1	0.2	18	0.010	4	266	0.016	50	16305	0.003	0.0	18	0.001	0.3	0	0.000	0.5	15	0.032	0.1	0.4	0.255
		D2	0.2	47	0.004	5	294	0.016	45	17101	0.003	0.0	19	0.002	0.3	0	0.000	0.6	233	0.003	0.1	0.0	1.318
		D3	0.1	27	0.004	4	315	0.013	37	16673	0.002	0.0	22	0.002	0.4	0	0.000	1.2	15	0.081	0.0	2.8	0.011
		D4	0.1	32	0.005	4	399	0.010	49	20104	0.002	0.0	51	0.000	0.5	21	0.021	1.2	30	0.040	0.1	0.5	0.177
	T2	D1	0.4	126	0.003	10	939	0.011	122	42472	0.003	0.8	300	0.003	1.1	304	0.004	1.1	14	0.079	0.4	0.2	1.733
		D2	0.2	122	0.001	8	615	0.013	47	34746	0.001	0.5	213	0.003	0.8	246	0.003	0.8	14	0.054	0.1	0.8	0.082
		D3	0.1	125	0.001	5	601	0.008	40	34012	0.001	0.2	106	0.002	0.4	225	0.002	0.6	12	0.052	0.0	7.8	0.001
		D4	0.1	131	0.001	3	817	0.004	39	38818	0.001	0.3	222	0.002	0.5	340	0.001	1.1	63	0.017	0.1	0.2	0.610
	T1	D1	0.3	67	0.004	37	770	0.048	104	39229	0.003	1.0	128	0.008	1.5	187	0.008	0.4	35	0.012	0.5	0.0	0.000
		D2	0.1	46	0.002	19	1432	0.014	37	49322	0.001	0.2	109	0.002	1.1	319	0.003	0.7	11	0.064	0.1	0.8	0.091
		D3	0.1	59	0.001	9	1413	0.006	33	45789	0.001	0.1	123	0.001	0.8	289	0.003	0.7	14	0.052	0.0	1.5	0.024
		D4	0.1	117	0.001	16	1281	0.012	51	48310	0.001	1.1	201	0.006	1.1	294	0.004	0.9	21	0.043	0.1	0.5	0.161
S. spontanium	**T3**	D2	0.2	0	1.438	1	199	0.006	28	14518	0.002	0.0	20	0.000	0.3	1	0.460	1.3	9	0.156	0.0	25	0.000
		D3	0.1	19	0.003	1	237	0.003	19	14754	0.001	0.0	22	0.000	0.2	10	0.018	0.9	13	0.068	0.0	0.0	0.000
		D4	0.2	40	0.005	2	127	0.015	51	17172	0.003	0.0	130	0.000	0.3	0	0.000	1.1	67	0.016	0.0	0.7	0.031
	T2	D2	0.2	119	0.002	4	753	0.006	64	37465	0.002	0.5	185	0.003	0.8	252	0.003	1.2	13	0.098	0.1	1.3	0.056
		D3	0.1	108	0.001	1	664	0.002	14	33181	0.000	0.2	147	0.001	0.4	395	0.001	0.7	11	0.063	0.0	0.0	0.000
		D4	0.1	125	0.001	2	722	0.002	28	32886	0.001	0.0	193	0.000	0.3	218	0.002	1.2	41	0.028	0.0	2.1	0.007
	T1	D2	0.1	144	0.001	5	646	0.007	34	41703	0.001	0.7	220	0.003	0.7	223	0.003	0.9	10	0.092	0.0	2.7	0.012
		D3	0.1	158	0.001	3	537	0.005	46	39767	0.001	2.3	219	0.010	1.0	268	0.004	1.4	18	0.078	0.0	1.6	0.029
		D4	0.2	175	0.001	4	800	0.005	77	43217	0.002	0.9	270	0.003	1.1	281	0.004	1.3	16	0.078	0.1	0.7	0.171
P. vittata	**T3**	D1	0.3	2	0.157	2	279	0.006	53	16948	0.003	0.0	25	0.001	0.3	0	0.000	0.4	13	0.034	0.1	0.7	0.075
		D2	0.2	31	0.005	1	311	0.003	31	18269	0.002	0.0	21	0.000	0.3	2	0.123	0.4	44	0.009	0.0	0.4	0.061
		D3	0.1	0	0.000	1	314	0.004	20	17433	0.001	0.0	27	0.000	0.2	5	0.043	0.5	205	0.003	0.0	0.0	0.000
		D4	0.1	45	0.002	1	362	0.004	33	19039	0.002	0.0	40	0.000	0.2	24	0.010	0.7	34	0.021	0.0	1.3	0.012
	T2	D1	0.3	149	0.002	8	646	0.013	77	39986	0.002	0.6	181	0.003	0.8	417	0.002	0.9	10	0.087	0.1	0.8	0.140
		D2	0.2	106	0.001	2	625	0.004	25	38300	0.001	0.4	206	0.002	0.5	311	0.002	0.7	16	0.041	0.0	1.2	0.027
		D3	0.2	137	0.001	2	798	0.002	27	37316	0.001	0.5	103	0.005	0.6	277	0.002	0.8	13	0.060	0.0	4.5	0.003
		D4	0.2	127	0.002	4	660	0.006	51	36843	0.001	0.8	201	0.004	1.2	231	0.005	1.1	21	0.052	0.1	0.7	0.113
	T1	D1	0.3	79	0.004	19	912	0.021	65	41383	0.002	1.2	130	0.009	1.5	202	0.007	0.5	2	0.262	0.1	2.1	0.062
		D2	0.1	87	0.001	11	989	0.011	36	45306	0.001	0.4	159	0.003	1.5	241	0.006	1.0	14	0.071	0.0	2.4	0.012
		D3	0.1	51	0.002	6	1438	0.004	54	46845	0.001	0.1	637	0.000	1.2	306	0.004	0.7	23	0.030	0.1	0.4	0.181
		D4	0.2	114	0.001	6	888	0.007	55	40157	0.001	1.2	208	0.006	1.1	257	0.004	0.7	16	0.046	0.1	0.6	0.115
C. compressus	**T3**	D1	0.4	0	0.000	5	286	0.018	105	18365	0.006	0.2	5	0.049	1.0	0	0.000	1.0	59	0.017	0.1	0.1	0.986
		D2	0.3	17	0.016	4	306	0.014	55	18385	0.003	0.1	25	0.005	1.2	16	0.077	1.1	79	0.014	0.1	0.3	0.202
		D3	0.1	12	0.011	3	294	0.012	39	16163	0.002	0.1	26	0.002	0.8	10	0.081	1.0	14	0.075	0.0	13	0.000
		D4	0.2	38	0.005	3	362	0.008	38	19281	0.002	0.0	44	0.000	0.8	17	0.044	1.2	37	0.033	0.0	3.3	0.003
	T2	D1	0.5	145	0.003	7	756	0.009	115	39431	0.003	0.6	238	0.003	1.0	327	0.003	0.9	5	0.200	0.2	0.8	0.288
		D2	0.3	163	0.002	5	721	0.007	43	39251	0.001	0.8	236	0.003	1.6	302	0.005	1.1	14	0.079	0.1	1.5	0.034
		D3	0.1	127	0.001	3	669	0.005	27	37175	0.001	1.1	188	0.006	0.7	250	0.003	0.8	10	0.080	0.0	3.9	0.005
		D4	0.2	143	0.002	6	952	0.007	44	39366	0.001	1.5	239	0.006	1.6	321	0.005	1.0	26	0.041	0.0	2.6	0.006
	T1	D1	0.3	71	0.005	38	966	0.040	119	44353	0.003	1.3	118	0.011	2.6	224	0.012	1.0	0	0.000	0.2	0.0	0.000
		D2	0.1	91	0.001	29	986	0.029	35	44373	0.001	1.7	138	0.012	2.8	244	0.011	1.7	10	0.175	0.1	3.0	0.020
		D3	0.2	83	0.003	18	1135	0.015	81	44771	0.002	1.4	168	0.009	2.7	224	0.012	1.2	8	0.151	0.1	1.9	0.044
		D4	0.2	132	0.002	8	659	0.012	81	41746	0.002	6.9	225	0.031	2.7	301	0.009	1.3	9	0.138	0.1	1.6	0.054