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RESEARCH ARTICLE

Vegetation composition and assessment of phytotoxicity in a paper mill dumpsite

Payal Das, Priyanka Bora, Nandita Paul & Nabanita Bhattacharyya*
Department of Botany, Gauhati University, Guwahati 781 014, Assam, India
**Email: nbh_17@gauhati.ac.in*

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ABSTRACT

The solid waste dumpsites of pulp and paper industries are prone to be turned into degraded lands due to the loss of vegetation cover. Such sites often possess drought, salinity and pH stresses as well as heavy metal contamination. Restoration of top soil by creating vegetation cover has proved to be the most sustainable approach to check land degradation. Therefore, to find some stress-tolerant species capable of creating vegetation cover in paper mill dumpsites, a vegetation composition study was conducted in a paper mill dumpsite. A total of seven plant species viz., *Calotropis gigantea* (L.) Dryand., *Chromolaena odorata* (L.) R.M. King & H. Rob, *Mikania scandens* (L.) Willd., *R. communis* L., *Rotheca serrata* (L.) Steane & Mabb., *Senna sophera* (L.) Roxb. and *Solanum myriacanthum* Dunal were found. To correlate the existence of these plants with stress condition of soil, the level of phytotoxicity in the dumpsite was assessed by studying seed germination status, proline accumulation, leaf relative water content (RWC), leaf pH, total chlorophyll content and ascorbic acid level of *Ricinus communis* as bioassay indices. The significantly lower percentage of seed germination in dumpsite soil, compared to control, revealed the phytotoxic nature of the soil of the dumpsite. The significantly higher level of proline, RWC, total chlorophyll and ascorbic acid in plant leaves from dumpsites than from the control soils indicated considerable stress in the dumpsite. Soil physicochemical and nutrient status analyses substantiated with the bioassay results. Despite apparent phytotoxicity, the presence of certain plant species in the dumpsite indicated their inherent stress tolerance capability to be prospected.

Introduction

Pulp and paper industries are among the most notorious environment degraders which discharge a variety of gaseous, liquid and solid wastes, including non-biodegradable organic materials, absorbable organic halogens, colour, phenolic compounds etc. into the environment (1). The lime sludges are among the main solid wastes generated by the paper mills and they are often disposed to nearby low-lying areas of the paper mills that form barren dumpsites without vegetation (2). Such areas are prone to be turned into degraded lands since the loss of plant cover is one of the significant causes of land degradation. Restoration of topsoil by creating vegetation cover is the most acceptable, sustainable and eco-friendly approach if one wants to prevent further degradation and recover the degraded areas into productive land masses for the benefit of the ever increasing population. Introduction of vegetation cover with proper management on a degraded land area may change the soil status of those areas (3). Selection of suitable and potential naturally growing indigenous plants with inherent capabilities to withstand the adversities of degraded lands is the foremost need of the hour in this respect.

Despite adverse physicochemical and biological properties of soil, some plant species can survive in the stressful condition of industrial and mining dumpsite with little agronomical effort. Information about such naturally growing plant species on degraded sites is crucial to create vegetation cover successfully on the dumpsites. Therefore, an attempt was made to study the composition of plants that were growing naturally in a paper mill dumpsite located in Assam, India aiming at finding more stress-tolerant species potential of creating vegetation cover on stress-laden paper mill dumpsites.

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However, assessment of stress and toxicity level in the particular site is required to infer about the stress tolerance potentiality of a particular plant species. Recently, the study of toxicity assessment in agricultural and urban soils based on bioassay methods are widely used in several countries (4–7). It is pertinent to use certain parameters like seed germination percentage and proline accumulation of some already established stress-tolerant plant species as bioassays to assess the toxicity level and stress burden of a particular site (7, 8). *Ricinus communis* L. (Euphorbiaceae) grows abundantly in harsh environmental conditions like industrial waste contaminated sites, along the roadsides, rail tracks, open lands and disturbed areas with high tolerance (9). This plant has been reported to be tolerant of different stresses like salinity, drought and frost (10). Comparative studies on different physiological and biochemical parameters of *R. communis*, grown in polluted and control condition, have been previously used as bio-monitoring parameters in a particular environment (11).

Therefore, in the present investigation, we have selected *R. communis* as a bio-indicator to study the stress and toxicity level in the aforesaid paper mill dumpsite. A hypothesis was set as "if *R. communis*, widely considered as stress-tolerant, shows a significant positive response towards the selected stress indices, then the dumpsite can be said under stress burden". Seed germination percentage, proline accumulation level, relative water content (RWC), leaf pH, total chlorophyll content and ascorbic acid level were selected as stress indices as previously described (12). Soil physicochemical properties and nutrition status of the dumpsite were also investigated to corroborate with the bioassay findings to confirm the stress of the site compared to control.

Materials and Methods

Study sites

For this study, we have selected the dumpsite of Nagaon Paper Mill (NPM), located in Morigaon district of Assam, India, at 26°4′ N and 92°5′ E (Fig. 1). The site is 60 km away from the state capital Guwahati. The temperatures of the region range between 30 °C to 37 °C during the summer months (June–July) and 8 °C to 22 °C during winter (December–January). The yearly average rainfall of the area is about 1399 mm.

For comparison with the dumpsite soils, a location in Gauhati University campus, located in Jalukbari, Guwahati, at 26°14′N and 91°73′E was selected as the control site. There is no apparent burden of pollution or any dumpsites in and around the area, and natural vegetation is covering the university campus. The location of the control site is about 60 km away from the dumpsite.

Vegetation composition in the dumpsite

The naturally growing plant species in the dumpsite were collected and herbaria were prepared for their identification according to standard procedure (13). Plant species were deposited in GUBH herbarium in the Department of Botany, Gauhati University.

Twenty 1 × 1 m^2 quadrats were placed randomly inside the dumpsite wherever plants were present. Individual species were recorded from the quadrats. The quadrat information from the plots was used to analyse compositional features like frequency (F), density (D) and abundance (A) (14), according to the following equations:

$$F\% = \frac{Number\ of\ quadrats\ of\ occurrence}{Total\ number\ of\ quadrats\ studied} x\,100$$

$$D = \frac{Total\ number\ of\ individuals\ of\ a\ species}{Total\ number\ of\ quadrats\ studied} x\,100$$

$$A = \frac{Total\ number\ of\ individuals\ of\ a\ species}{Number\ of\ quadrats\ of\ occurrence}$$

Estimation of stress indices in R. communis

Proline content in the leaf tissue was analysed according to the standard method (15). Five replicates were made for each plant sample collected from the dumpsite and control site.

For the determination of RWC, leaves were collected in the morning hrs (9.00 am to 11.30 am) from the selected plants from the sites in triplicate. RWC was calculated using the following formula (12):

$$RWC = \frac{Wf - Wd}{Wt - Wd} x\,100$$

Where *Wf* = fresh weight of leaf

Wt = turbid weight of leaf

Wd= dry weight of leaf

Leaf pH was determined, as described earlier (16). About 0.5 gm of leaf tissue was ground to paste, mixed in 50 ml of distilled water and then filtered. After that, the pH of the filtrate was determined by using a calibrated digital pH meter.

Total chlorophyll content analysis was done according to the standard method (17) and expressed as mg/gm of fresh weight.

$$Total\ chlorophyll = [20.2(A645) + 8.02(A663)]\, x\, \frac{V}{1000\, xW}$$

Where,

A = absorbance at specific wavelengths

V =final volume of chlorophyll extract in 80% acetone

W =fresh weight of tissue extracted

Ascorbic acid content was spectrophotometrically determined (18) by calculating from the standard curve of known ascorbic acid.

Seed germination assay of *R. communis* seeds was done according to the methodology described earlier (19). Seeds were collected from Regional Sericulture Research Station (RSRS), Boko, Kamrup, Assam. Five seeds were sown for germination in a plastic cup of 5 cm diameter and 10 cm length, filled with 200 gm of soil. Three replicates were made for

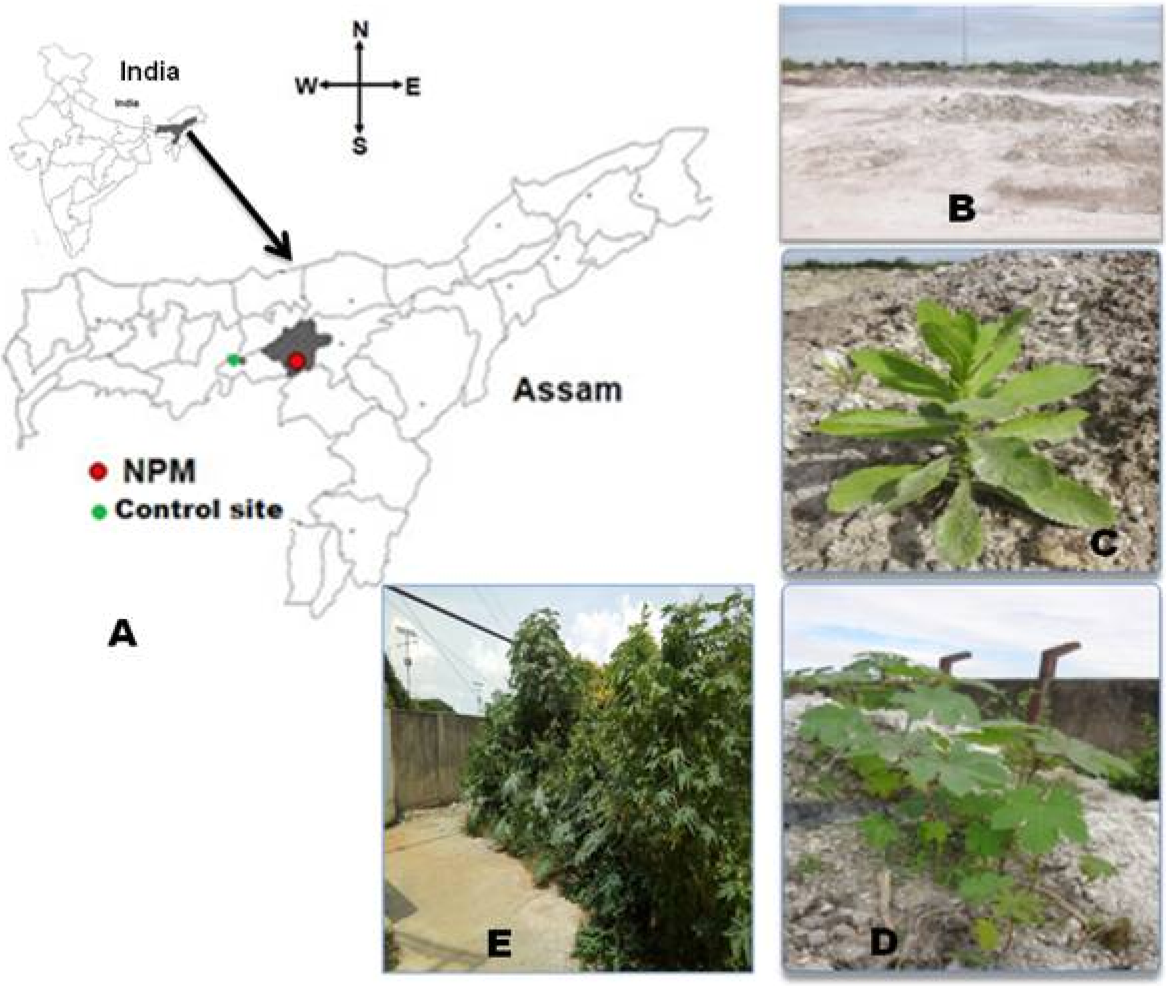

Fig. 1. **A**=Map of India and Assam showing the study area (https://d-maps.com) **B**=Photo of the barren dumpsite; **C, D**=Photos of the dumpsite with some plants [C=*R. serrata*; D=*R. communis*] **E**= The control site.
[Source of map: https://d-maps.com/carte.php?num_car=31173&lang=en]

soil collected from the dumpsite and control site. Seeds were sown on the surface of the soil first and then covered with 1 cm of the soil layer and kept up to 10 days for germination with regular spraying of water. The seed germination percentages were recorded on the 10th day by using the following formula:

$$\%\ of\ germination = \frac{Number\ of\ seeds\ germinated}{Number\ of\ total\ seeds\ sown} x\ 100$$

Soil physicochemical parameters and nutrient status

Five replicates of soil samples were collected in clean polypropylene bags from 10–15 cm depth by digging the soil in V- shape with the help of a spade. The soil samples were then air dried by spreading on clean sheets of papers after breaking the large lumps and finally they were sieved through 2 mm sieves for further laboratory analyses.

Soil classification and texture analysis were done by following the standard method (20). For soil moisture, 5 gm of soil samples were taken in Petri dishes and kept in a hot-air oven (50 °C) for 1 day. Next day, the weight of the soil samples were measured and then again kept for another day. This was repeated until the weight of the soil sample gave a constant measurement. The final weight of the soil samples was subtracted from the initial weight of the soil to get the accurate amount of soil moisture content and expressed as percentage soil moisture.

The analyses of other soil physicochemical parameters including soil pH, electrical conductivity (EC) and soil nutrient status including soil organic carbon (OC) and other macro and microelements of the soil, eg., nitrogen (N), phosphorus (P), potassium (K), sulphur (S), zinc (Zn), boron (B), iron (Fe), manganese (Mn) and copper (Cu) were performed in a mini lab viz., *Mridaparikshak* in the laboratory of Soil Science Department of Central Muga Eri Research and Training Institute (CMERTI), Lahdoigarh, Jorhat, Assam. This mini lab was developed by ICAR- Indian Institute of Soil Science (IISS), Bhopal under the division of Natural Resource Management of Indian Council of Agricultural Research and is being widely used to test soil parameters (21, 22). All the parameters were analysed using the solutions or reagents supplied as a kit, according to the instructions by ICAR, provided along with the instrument. Calcium (Ca) and magnesium (Mg) of soil extract was measured with the EDTA-titrimetric method (23).

Data analysis

All the data presented are the mean of three replicates with standard deviation. The data were analysed by Microsoft Excel programme and by the method of Snedecor and Cochran (24). Significance tests were done with the help of statistical software program SPSS with significance level of $p < 0.05$.

Results and Discussion

Compositional features of plants in dumpsites

A total of seven plant species were found in the dumpsite, which included *Calotropis gigantea* (L.) Dryand., *Chromolaena odorata* (L.) R.M. King & H. Rob, *Mikania scandens* (L.) Willd., *Ricinus communis* L., *Rotheca serrata* (L.) Steane & Mabb., *Senna sophera* (L.) Roxb. and *Solanum myriacanthum* Dunal. Number of plants were very few accounting for a very low overall stand density which was 1.8. *R. communis* was found to be grown comparatively more luxuriantly. The relative contribution of the species to overall stand density was 33.33 % which was higher than those of other associates. The decreasing order of relative density of the recorded plants were *R. communis* > *S. sophera* > *S. myriacanthum* > *M. scandens* > *C. gigantea* > *R. serrata* and *C. odorata*. The density of *R. communis* was the highest (0.6) among all other species found in the dumpsite. The frequency of occurrence was also highest (50%) in the case of *R. communis* (Table 1).

R. communis has been widely considered ideal for remediation and to improve green cover as it stabilises the nutrient cycling (9). However, detailed literature is lacking on the role of other six plants in a natural succession of paper mill dumpsites. Previously, *C. odorata* was demonstrated to have phytoremediation potential to remediate heavy metals (25), used engine oil (26) and polychlorinated biphenyls (PCB) (27) from contaminated soil. Therefore, this plant can be successfully tried as a promising candidate for the removal of heavy metals and toxic chemical compounds from the soil of paper mill dumpsites for rehabilitation.

All these plants those were observed in the dumpsite had great ethnomedicinal and economic importance as per available literature (Table 2). This is promising for the optimum use of degraded sites for the economic development of the local people by implementing favourable agronomic practices.

Bioassay indices in R. communis

Proline content

Proline content of *R. communis* leaf was significantly higher ($P < 0.05$) in dumpsite (63.76 ± 2.54 µg/g) as compared to the control site (58.08 ± 1.03 µg/g) (Fig. 2). Higher proline can be attributed to increased soil salinity as evidenced by higher EC, which was also demonstrated earlier (30). The increase of proline in plant cells is a physiological response of plants to adversity which can be considered as the indicator of the relative resistance of plants against stress (12). Hence, a significant increase in proline level in the samples from the dumpsite than in those from the control site points out that the site has considerable stress burden.

Leaf RWC and pH

Leaf RWC of *R. communis* from dumpsite was found to be 58.52 ± 0.85 % while it was 48.92 ± 0.83 % in the samples from the control site that was significantly different ($P < 0.05$) (Table 3). High water content within a plant body helps to maintain its physiological balance under stress condition (31). Thus, high RWC in the leaves of a stress-tolerant plant is an indicator of stressed condition encountered by the plant. Generally, RWC decreases in plants which are sensitive to stress with stress progression (12, 32). However, high RWC in stress-tolerant plants in response to increased stress level is considered to be an adaptive feature necessary for the successful establishment of the plant in areas under certain stress burden (33–36). Thus, a significantly higher percentage of RWC in the leaves of *R. communis* from the dumpsite than that from the control site, despite the insignificant difference in soil moisture between the two sites, reveals considerable stress level in the dumpsite.

Table 1. Compositional features of naturally growing plants in the dumpsites

Name of the plant	Family	Frequency (F)	Abundance (A)	Density (D)	Relative Density	A/F
Calotropis gigantea (L.) Dryand.	Asclepiadaceae	15	1	0.15	8.33	0.067
Chromolaena odorata (L.) R.M. King & H. Rob	Asteraceae	10	1	0.1	5.56	0.1
Mikania scandens (L.) Willd.	Asteraceae	10	2	0.2	11.11	0.2
Ricinus communis L.	Euphorbiaceae	50	1.2	0.6	33.33	0.024
Rotheca serrata (L.) Steane & Mabb.	Lamiaceae	10	1	0.1	5.56	0.1
Senna sophera (L.) Roxb.	Fabaceae	30	1.33	0.4	22.22	0.044
Solanum myriacanthum Dunal.	Solanaceae	20	1.25	0.25	13.89	0.063
				Total = 1.8		

In other studies, *C. odorata, C. gigantea, S. sophera* and *M. scandens* were reported to be growing naturally as dominant species among others, in peripheral as well as core zones of various coal mine overburden dumps of India (28, 29). Therefore, presence of these plants naturally in the dumpsite may be suggested as an initial step of succession towards remediation through the process of revegetation to restore the favourable and stable conditions in the site for sustainable land use in future.

The leaf pH of dumpsite sample was found to be 5.27 ± 0.42, which was significantly lower ($p < 0.05$) than the control site sample with leaf pH 7.34 ± 0.05 (Table 2). The pH is a stress-responsive parameter that acts as an indicator for various pollution sensitivity, as reported previously (16). Therefore,

Table 2. Ethno-medicinal and economic importance of plants from paper mill dumpsites

Botanical Name	Medicinal value	Reference
Calotropis gigantea (L.) Dryand.	Used against asthma, toothache, earache, swelling, inflammation in sprain	(62)
Chromolaena odorata (L.) R.M. King & H. Rob	Useful as anti-inflammatory, astringent, diuretic and hepatotropic activities, antimicrobial, enhancement of antibiotic activity, antioxidant etc.	(63)
Mikania scandens (L.) Willd.	Used to treat stomach ulcers, gastric problems.	(64)
Ricinus communis L.	Oil and seed extracts are used in severe constipation, worm infestation, rheumatism, intestinal inflammation, boils, headaches, inflammatory skin diseases and birth control.	(9)
Rotheca serrata (L.) Steane & Mabb.	Useful as acrid, thermogenic, anti-inflammatory, digestive, carminative, stomachic, anthelmintic, depurative, expectorant, sudorific and stimulant.	(65)
Senna sophera (L.) Roxb.	Useful in epilepsy, ascites, dyscrasia of liver, skin disorders, piles, jaundice, fever, articular pain, palpitation, asthma, acute bronchitis, cough, diabetes and convulsions of children.	(66)
Solanum myriacanthum Dunal.	Used in the folk medicine of Tangkhul Naga tribe of India for treating intestinal worms.	(67)

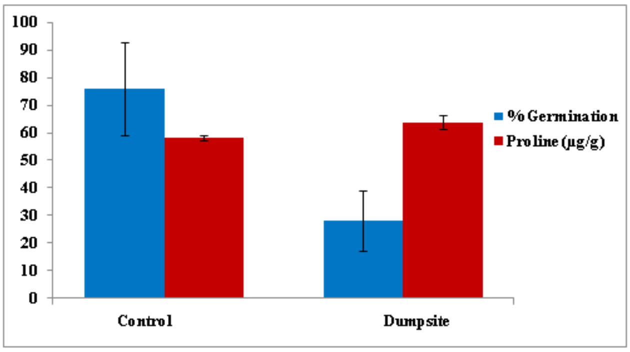

Fig. 2. Seed germination and proline content of *R. communis* collected from different sampling sites.

significantly low leaf pH in *R. communis* of the dumpsite indicates the presence of stress in the site.

Table 3. Different stress indices estimated in *R. communis* with (±) standard deviations

Study sites	RWC (%)	Leaf extract pH	Total chlorophyll (mg/g of fresh weight)	Ascorbic acid (mg/g of fresh weight)
Dump Site	58.52 ± 0.85	5.27 ± 0.42	0.238 ± 0.02	2.53 ± 0.35
Control Site	48.92 ± 0.83	7.34 ± 0.05	0.171 ± 0.01	1.35 ± 0.14

Chlorophyll content

Total chlorophyll content in the leaves of *R. communis* from the dumpsite was estimated as 0.238 ± 0.02 mg/gm of fresh weight which was significantly higher ($p < 0.05$) than the control site, *i.e.* 0.171 ± 0.01 mg/g (Table 3). Previous studies revealed that stress-tolerant lines of plants showed increased chlorophyll content in response to different stresses (37–39). This responsive trend is also obvious in *R. communis* as an adaptive strategy against various levels of stresses (40, 41). Therefore, the significantly higher value of chlorophyll in the sample from dumpsite suggests the stress burden in the site.

Ascorbic acid content

Ascorbic acid was found to be 2.53 ± 0.35 mg/gm of fresh weight in samples from the dumpsite which was significantly higher ($p < 0.05$) than that in samples from the control site, ie. 1.35 ± 0.14 mg/gm of fresh weight (Table 3). Ascorbic acid is a well studied non-enzymatic antioxidant which has a prominent role in scavenging reactive oxygen species (ROS) and in modulating various functions in plants, especially under stress. Increase in accumulation of ascorbic acid increases tolerance to oxidative, salt and drought stresses in several species, as shown in earlier reports (42-48). Thus, the significantly higher ascorbic acid content in the sample from dumpsite indicates that the site is under considerable stress for plant growth.

Seed germination

R. communis showed 76 ± 16.73 % seed germination in control and 28 ± 10.95 % in dumpsite soil (Fig. 2). We found the seed germination percentage in soil collected from dumpsite to be significantly lower than in soil collected from the control site ($P<0.05$). The decrease in seed germination percentage corroborated with many other studies, where germination percentages of different plant species decreased with an increase in paper mill effluent concentration (49–53).

Seed germination test has got quite an attention as a simple and inexpensive bioassay for detection of toxic elements in soil, compost etc. (54). Previous studies suggested *R. communis* as a promising species as a bio-monitor of atmospheric pollution because of its wide distribution across the globe and in harsh condition (55). Therefore, low germination percentage of *R. communis*, which is otherwise a well-reputed stress-tolerant plant, indicates the considerable phytotoxicity in the dumpsite soil.

Soil physicochemical properties

In the present study, soil texture of the dumpsite was found to be silty clay loam type with 60% silt, 28% clay and 12% sand, whereas soil texture in controlsite was of clay loam type. The total moisture contents of the dumpsite and the control site were not significantly different ($p>0.05$) (Table 4). However, the soil pH of the dumpsite was found to be significantly higher (8.814 ± 1.09; $p< 0.05$), than the control site (5.15 ± 0.40) (Table 4). Previous studies showed that alkalisation deteriorated the availability of phosphorus and micronutrients, which could negatively affect plant growth in various ways (56).

The electrical conductivity of the dumpsite soil was also found to be significantly higher ($p < 0.05$) compared to the control site indicating that the soil of dumpsite is more saline than the control site. This might be because of the leakage of ionically rich effluent of the paper mill containing an extensive amount of chemicals used in the different processing units (57).

Soil nutrients like OC, N, P, K, S, Zn, B, Fe, Mn, Cu, Ca and Mg showed significantly higher values ($p<0.05$) than the control site (Table 4), which substantiated several previous studies on the effect of paper mill effluent on soil properties (58). Significantly higher values of these elements may be attributed to the high nutrient and heavy metal rich effluent released from the paper mill (57, 59). Lime sludge, one of the main solid wastes generated by the paper mill that is disposed to that site, would be one reason of high Ca in the degraded soil of dumpsite. Although Ca and Mg are essential for the growth and development of plants, the higher level of them leads to alkalinisation of soil by altering the ionic balance in soil (60). Previous studies suggested that the excess of Ca in soil was more harmful than soil salinity for plants' health (61).

Accumulation of high amounts of various organic and inorganic materials and toxic trace elements after long-term deposition possibly resulted in nutrient overload, heavy metal contamination and subsequent degradation of soil in the dumpsite, as revealed by an earlier study (58).

Table 4. Soil moisture contents control and dumpsites.

Soil parameters	Control site soil sample	Dumpsite soil sample	Permissible limit (68, 69)
Soil moisture (%)	48.8 ± 0.32	49.7 ± 0.88	NGVS
Soil pH	5.15 ± 0.40	8.814 ± 1.09	4-8.5
Electrical Conductivity (EC) (dS/m)	0.114 ± 0.02	0.438 ± 0.24	4 dS/m
Organic Carbon (OC) (%)	1.17 ± 0.04	1.618 ± 0.42	NGVS
Nitrogen (N) (kg/h)	415.78 ± 4.89	571.72 ± 9.72	NGVS
Phosphorus (P) (kg/h)	23.92 ± 1.29	30.26 ± 3.61	>14 kg/h
Potassium (K) (kg/h)	335.66 ± 11.9	538.76 ± 19.5	>160 kg/h
Sulphur (S) (mg/kg)	32.90 ± 2.35	76.62 ± 7.14	NGVS
Zinc (Zn) (mg/kg)	9.72 ±0.25	15.96 ± 0.79	250 mg/kg
Boron (B) (mg/kg)	1.18 ± 0.09	4.59 ± 0.27	NGVS
Iron (Fe) (mg/kg)	22.34 ± 0.64	39.68 ± 4.41	NGVS
Manganese (Mn) (mg/kg)	21.18 ± 1.37	46.48 ± 1.44	500 mg/kg
Copper (Cu) (mg/kg)	20.96 ± 0.89	34.92 ± 3.01	100 mg/kg
Calcium (Ca) (mg/kg)	551.3 ± 4.16	1899.3 ± 5.03	NGVS
Magnesium (Mg) (mg/kg)	116.6 ± 0.30	408.4 ± 1.83	NGVS

NGVS= No guideline value set

Conclusion

Phytotoxicity analysis based on proline accumulation, leaf RWC, leaf pH, chlorophyll content and ascorbic acid content and seed germination percentage of one of the established biomonitoring model plant *R. communis* has shown that the NPM dumpsite was phytotoxic in nature. The results of phytotoxicity study have been well substantiated by the soil physicochemical parameters analysed in the laboratory, thus suggesting the stress burden and toxicity in the dumpsite. Despite the harsh condition for vegetation growth, some plants, with great ethnomedicinal and economic importance, were found to be grown naturally in the site. Some of these plants have also been reported previously among tolerant natural vegetation in other industrial and mining dumpsites. Such plants might have inherent capabilities to create better vegetation cover without much effort, if proper management procedures are followed, to recover the dumpsites into arable lands in due course of time. However, the selection of the plants should be made perceptively to restore the natural dynamics of the ecological succession logically. Further monitoring of the dumpsite is important to note the changes in the habitat conditions in the natural course of succession to make the site suitable for the establishment of the desired group of plants in the future.

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Authors' contributions

PD carried out the soil sample collection and analysis as well as collection and identification of plants from the dumpsite. PB carried out the collection of plant materials, estimation of chlorophyll, RWC, Ascorbic acid and leaf pH. NP took part in the collection of plant materials, seed germination assay and proline estimation of leaf samples. NB conceived the concept of the study, design and coordination, performed the statistical analysis and drafted the manuscript. All authors read and approved the final manuscript.

Conflict of interests

Authors do not have any conflict of interests to declare.

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