

1 Screening of Heavy Metal Tolerant Jute Seeds by Germination Test

3 Abstract

4 Present study was the part of an ongoing green plant based contaminated soil remediation
5 technology. The experiment was conducted in two phases. The 1st phase of the experiment was
6 carried out in the laboratory of the department of Agricultural Chemistry, Bangladesh
7 Agricultural University, Mymensing with a view to screening different varieties of jute seeds
8 against the single and combined toxicity of heavy metals viz. As, Pb, Cd by germination test to
9 select heavy metal tolerant jute seeds. Initially 10 treatments were considered viz. 0.5, 10, 15, 20,
10 30, 50, 70, 100 and 150 ppm for As, Pb, Cd separately and in combination with As, Cd and Pb.
11 The increased levels of heavy metals significantly decreased the germination percentage,
12 seedling height, shoot and root length, fresh and dry weight of five jute varieties. Among five
13 varieties height germination percentage seedling height, shoot and root length, fresh and dry
14 weight were observed with BINA deshi pat-2 but the seedlings survived up to 100 ppm single and
15 combined doses of As, Cd and Pb. Germination and seedling vigor of other four varieties were
16 much less resistance of heavy metal treatments. After screening, BINA deshi pat-2 was used as a
17 phytoremediant in the second phase of bioremediation process which such carried out in the net
18 house from October 2009 onwards. Other two varieties namely BADC deshi pat (CVL-1) and
19 atom pat-38 were selected from experiment conducted by other researcher. In the 2nd phase
20 number of treatments were reduced to six from ten such as 0, 15, 40, 70, 100 and 150 ppm single
21 treatments of the experiment revealed that, seeds of the three varieties germinated in toxic soil
22 environment but their primary growth was not satisfactory. Reasons behind the stunted growth
23 might be due to off-season and unfavorable environmental condition. But our idea was to grow
24 jute in successive seasons in a year. BINA deshi pat-2 though photosensitive variety but in pot
25 trial in off season from October, 2009 onwards failed to grow at minimum height not only at
26 toxic condition but also in non toxic soil i.e. in control treatment.

27
28 **Keywords:** Heavy Metal, Jute Seeds and Germination Test

30 Introduction

31 Contamination of agricultural soil by heavy metals viz. As, Pb, Cd, Cr is a great concern in
32 Bangladesh and as well as over the globe. The term heavy metals indicate metal having density
33 more than five times than that of water, air, plant and animals in their tissue.

34 Arsenic (As) is a toxic heavy metal, widely encountered in the environment, ecological
35 component and abundance in the earth crust. Arsenic contamination in Bangladesh is probably
36 geological in nature, originating from the fine alluvial sediments of the Ganges Delta.

37 In Bangladesh arsenic contamination of ground water was first confirmed in 1993 at
38 ChapaiNawabganj district. At present 59 districts across the country are affected by arsenic
39 poisoning. Consequently 80 million people are new exposed to the threat of arsenic and 10,000
40 people have shown the symptoms of arsenicosis(Zamanet *al.*, 2005).

41 According to the World Health Organization (WHO,1999)about 80 million people in Bangladesh
42 are threatened by arsenic poisoning.

43 The oxidation of arsenopyrite or ferrous hydroxide minerals may be responsible for the release of
44 arsenic oxide in solution to the groundwater. Groundwater contamination by Arsenic (As) in
45 Bangladesh appears to be the longest mass poisoning in the world. Arsenic can cause
46 contamination in surface soil through arsenic contaminated groundwater irrigation which in term
47 enhances the levels of soil arsenic. The presence of high concentration arsenic in surface soil
48 may result in high concentration of arsenic in cereals, vegetables and agricultural product and
49 contaminate the food chain affecting human health. According to WHO (1999), 0.01 mg As for
50 drinking water, 0.2 mg As L⁻¹ for livestock, 0.1 mgAs L⁻¹ for irrigation are recommended
51 where 100 times more than that in different regions are found. High concentration of Cd in soil
52 represents a potential threat to human health because it is incorporated in the food chain mainly
53 by plant uptake (Alvarez-Ayuso, 2008).

54 The toxicity of heavy metal is a part of ecological, evolutionary and environmental reasons
55 (Nagajyotiet. *al.*, 2008). Germination of seed is the vital phase for successful crop production. If
56 germination is affected in any way thenproduction will fall drastically. Negative effects of Pb
57 toxicity on seed germination and seedling growth were examined (Iqbal *et. al.*, 2004). Lead (Pb)
58 produced highly significant effects on shoot, root lengths and dry biomass of *Lythrumsalicanea*
59 (Joseph *et. al.*, 2002).Presence of excess amount of heavy metal in the growth media affects seed
60 to germinate and subsequent seedling growth. To meet the challenge of food security of 21st
61 century research emphasis should be given on pollution free water and soil of Bangladesh.
62 Heavy metal contaminated underground water is randomly used for irrigation in Bangladesh
63 agriculture which ultimately pollutes the soil. Growing of edible crops in contaminated soil is of
64 ultimate threat to the human health as well as livestock. So, emphasis should be given on
65 remediation of toxicant from contaminated soil is brought under cultivation by non-edible crops.
66 This income generating technology will clean the heavy metal contaminated soil as well as
67 farmers will be economically benefited.

68 Research work relating this topic is very scanty in the world and a well as in Bangladesh.
69 However, an effort has been made to conduct a study with the following major objectives:

- 70 i) To screen different varieties of jute seeds against single and combine toxicity of heavy
71 metal by germination test.
- 72 ii) To observe the germination and seedling growth of different varieties of jute seeds in
73 heavy metal contaminated soil in pot culture.

74 **Materials and Methods**

75 **3.1 Experimental site:**

76 **The experiment** was conducted in two phases. In first phase, screening of heavy metal tolerant
77 different varieties of jute seeds was done by germination test in the laboratory of the Department
78 of Agricultural Chemistry, Bangladesh Agricultural University, Mymensing from July to August,
79 2009.

80 After screening the jute seeds as phyto-remediants the second phase of the experiment was
81 conducted in the net house of the Department of Agricultural Chemistry, Bangladesh
82 Agricultural University, Mymensing, from October 2009 onwards.

83 **3.2 Test Seeds:**

84 Following five different varieties of jute seeds were used to germination test under increased
85 levels of single and combined toxicity of arsenic (As), lead (Pb) and cadmium (Cd).

- 86 a) Tosh pat (0-9897)
87 b) BINA deshi pat-2
88 c) 400 GYP-15(2)
89 d) 400 GYP-65(2)
90 e) 400 GYP-198(2)

91 **3.3 Experimental procedure of the first phase of the experiment:**

92 Germination test of different varieties of jute seeds were conducted in this phase under different
93 levels of single and combined toxicity of heavy metals viz. As, Cd and Pb. Ten (10) different
94 treatments were used such as 0.5, 10, 15, 20, 30, 50, 70, 100 and 150 mgL⁻¹ separately for As,
95 Pb, Cd and combination of As, Cd and Pb. Twenty seeds of each test cultivars were placed in
96 each petridish filled with cotton. Desired concentration of As, Cd and Pb were calculated from
97 sodium arsenate (NaAsO₂), Cadmium nitrate [Ca(NaO₃)₂ 4H₂O] and lead nitrate [(Pb(NaO₃)₂]
98 respectively. 10 ml of test solution was placed in each petridish. The cotton was kept moist
99 constantly with distilled water. The experiment was arranged in a Completely Randomized
100 Design (CRD) with three replications at room temperature (28±1°C). Data were recorded after
101 seven days from sowing the seeds in petridish.

102 **3.4 Parameter studied:**

103 Studied in this phase:

- 104 a) Number of seed after 7 days
- 105 b) Germination percentage (%)
- 106 c) Seedling height (cm)
- 107 d) Shoot length (cm)
- 108 e) Root length (cm)
- 109 f) Fresh weight (g)
- 110 g) Dry weight (g)

111 **3.5 Experimental data:**

112 Experimental data were collected on the following parameters:

113 **3.5.1 Seed germination:**

114 Germinated seeds were counted from the beginning of the seed germination up to complete
115 germination.

116 **3.5.2 Seedling height (cm):**

117 The plant height was recorded at the time of germination. The height was measured from the
118 internal base of petridish to tip of the main stem in cm.

119 **3.5.3 Shoot and Root length of seedlings:**

120 The shoot and root length of seedlings were measured after 7 days of the respective
121 germination setting.

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123 **3.5.4 Fresh weight of seedlings:**

124 After 7 days of seedlings were harvested and immediately after harvesting fresh weight of the
125 seedlings were recorded.

126 **3.5.5 Dry weight of seedlings:**

127 The dry weights of seedlings were measured after 7 days of the germination settings.
128 Immediately after harvesting seedlings were sun dried followed by oven drying, the actual
129 weight was measured thereafter.

130 **3.6 Statistical analysis:**

131 The collected data on various parameters were statistically analyzed. The means for all
132 treatments were calculated and analysis of variance for all chapters performed by F-test. The
133 significance of difference between the pairs of means was calculated by Duncan's Multiple
134 Range Test (Gomez and Gomez, 1984).

135 **3.7 Experimental procedure in the second phase of the experiment:**

136 After screening desired varieties viz. BADC deshi pat (CVL-1), BINA deshi pat-2 and Atom
137 pat-38 were used as a phytoremediant in the second phase of the study. Experimental
138 procedure is as follows:

139 **3.7.1 Collection and preparation of soil:**

140 For second phase of the study soil was collected from the surroundings of the KarimBhawan
141 of BAU, campus at 0-15 cm depth.

142 **3.7.2 Pot preparation:**

143 An amount of 15 Kg soil was taken in a series of plastic pot each pot was 30 cm deep with 27
144 cm diameter at the top and 22 cm diameter at the bottom. Top surface area of each pot was 22
145 cm². The total no of pots used in this study was 36.

146 **3.7.3 Treatments:**

147 In the second phase of the experiment, treatment numbers were reduced from ten to six viz. 0,
148 15, 40, 70, 100 and 150 mgkg⁻¹ soil separately for As and combination of As, Cd and Pb.
149 Urea, TSP, MoP and Gypsum were added at the rate of 135 kgha⁻¹, 100 kgha⁻¹, 70kgha-1
150 respectively each pot. Treatment was reduced to get intensively experimented data. Above
151 recommended doses of fertilizer were applied three times in the soil.

152 **3.7.4 Intercultural operations:**

153 Necessary intercultural operations such as weeding, watering, fertilizing and pesticide
154 application were done as and when needed.

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Results and Discussion

158 **4.1 Effect of heavy metal on germination of different varieties of jute seeds**

159 **4.1.1 Effect of arsenic on germination**

160 Increased levels ofAs significantly decreased the percentage of germination against the seeds
161 of all the cultivar under test. Response of As on germination were identical at 5ppm As
162 treatment and in control in case of Tosha pat (0-9897) and 400Gy P-15(2). Seeds of rest of the
163 varieties showed negative effect at 5ppm As and onwards. The trend of germination reflected
164 that the seeds of 400 Gy P-198(2) showed 0 percent germination at 70ppm As followed by
165 500 Gy P-65(2) at 100ppm As. But at 150ppm As treatment not a single seed of any variety

166 germinated. With the increasing levels of As germination percentage gradually declined in all
167 five varieties.

168 **4.1.2 Effect of lead on germination**

169 A significant negative relationship was obtained lead and germination of different varieties of
170 jute seeds. Germination percentage ranged from 70-0, 75-0, 70-0, 70-0 and 65-0 in case of
171 Tosha pat (0-9897), BINA deshi pat-2, 400 GyP-15(2), 500 Gy P-65(2) and 400 Gy P-198(2)
172 variety respectively. Highest germination (15) and germination percentage (75) was found in
173 case of BINA deshi pat-2 variety at controlled treatment while lowest was observed in Tosha
174 pat (0-9897) and BINA deshi pat-2 variety at 100ppm of lead.

175 **4.1.3 Effect of cadmium on germination**

176 Negative impact of cadmium on germination of different varieties of jute seeds. Variety BINA
177 deshi pat-2 and line 500Gy P-65(2) showed decreasing trend in case of germination. The
178 highestgermination percentage was found inBINA deshi pat-2 (75%) followed by Tosha pat
179 (0-9897), 400 Gy P-15(2) and 500 Gy P-65(2) having 75% germination at controlled
180 treatment. Reason behind the decreasing trend of germination might be due to the detrimental
181 effect of cadmium on physiology and cell division of jute seeds.

182 **4.1.4 Combined effect of arsenic, lead and cadmium on germination**

183 Germination of different varieties of jute seeds significantly decreased due to the combined
184 toxicity of arsenic, lead and cadmium. Among the five varieties only BINA deshi pat-2 has
185 showed its highest level of combined toxicity tolerance capacity at 100ppm arsenic, lead and
186 cadmium. In fact the highest (60) and lowest (5) germination percentage was found in BINA
187 desh pat-2 variety at 0 ppm and 100 ppm concentration respectively.

188 **4.2 Effect of heavy metal on seedlings height of different varieties of jute**

189 **4.2.1 Effect of arsenic on seedlings height**

190 Seedlings height decreased gradually with increased level of arsenic concentration. Seedlings
191 height ranged from 5.9-0, 4.2-0, 4.3-0 and 4.2-0 for Tosha pat (0-9897), BINA deshi pat-2 and
192 400 Gy P-15(2); 500Gy P-65(2) and Gy P-198(2) respectively. Highest (5.9 cm) and lowest
193 (0.7 cm) seedling height was obtained both in Tosha pat (0-9897) variety at 0 ppm and 100
194 ppm arsenic concentration respectively.

195 **4.2.2 Effect of lead on seedlings height**

196 Increased level of lead concentration gradually refunded the seedlings height of different
197 varieties of jute seeds as showed in the table 1. Among the 5 varieties the tallest seedling (5.1
198 cm) was obtained in the control treatment of BINA deshi pat-2 variety. While the lowest
199 seedling height (0.7 cm) was found in Tosha pat (0-9897) at 100 ppm Pb concentration.

200 4.2.3 Effect of cadmium on seedlings height

201 Unlike arsenic and lead increased level of cadmium toxicity gradually declined the seedlings
202 height of different varieties of jute seeds. Among the 5 varieties the tallest seedling (4.9 cm)
203 was obtained in BINA deshi pat-2 variety at controlled treatment. While the lowest seedling
204 height (0.7 cm) was obtained in Tosha pat (0-9897) at 100 cadmium toxicity. Seedlings height
205 ranged from 5.7-0, 4.8-0, 4-0, 4.8-0, and 4.8-0 for Tosha pat (0-9897), BINA deshi pat-2, 400
206 Gy P-15(2), 500Gy P-65(2) and 400 Gy P-198(2) respectively.

207 4.2.4 Combined effect of arsenic, lead and cadmium on seedlings height

208 Seedlings height of different varieties of jute seeds drastically declined due to combined
209 toxicity of arsenic, lead and cadmium. The highest seedling height (4.7 cm) was observed in
210 Tosha pat (0-9897) and BINA deshi pat-2 variety both at controlled treatment. While the
211 lowest seedling height (0.7 cm) was obtained in 500 Gy P-65(2) at 70ppm combined toxicity.
212 Seedlings height ranged from 4.7-0, 4.7-0, 3.7-0, 4.1-0 and 1.8-0 for Tosha pat (0-9897),
213 BINA deshi pat-2, 400 Gy P-15(2), 500 Gy P-65(2) and 400 Gy P-198(2) respectively. Reason
214 behind such declined trend might be due to combined toxic effect of As, Pb and Cd on
215 physiology and cell division of jute seedlings.

216 4.3 Effect of heavy metal on shoot and root length of different varieties of jute seeds

217 4.3.1 Effect of arsenic on shoots and roots length

218 Shoots and roots length also significantly decreased with increasing level of arsenic
219 concentration. The reason behind such declined trend might be due to combined toxic effect of
220 As, Pb and Cd on physiology and cell division of jute seedlings.

221 4.3.2 Effect of lead on shoots and roots length

222 A significant response in respect of shoots and roots length of different varieties of jute
223 seedlings was observed at 1% level of probability. Shoots and roots length decreased
224 gradually with the increase of Pb concentration (Table 2).

225

226 Table 1. Effect of Arsenic on shoots and roots length of different varieties of jute seeds

Varieties	Tosha pat 0-9897		BINA deshi pat-2		400 Gy P-15(2)		500 Gy P-65(2)		400 Gy P-198(2)	
	Shoot length (cm)	Root length (cm)	Shoot length (cm)	Root length (cm)	Shoot length (cm)	Root length (cm)	Shoot length (cm)	Root length (cm)	Shoot length (cm)	Root length (cm)
Treatments (mgAsL ⁻¹)										
0	5.6a	1.9a	4.6a	1.95a	4.0a	1.90a	4.0a	1.9a	3.9a	1.9a
5	5.0b	1.8ab	4.6a	1.85b	3.9a	1.85b	3.8a	1.8a	3.6a	1.8a

280 In a column figures with dissimilar letters differ significantly according to DMRT

281

282 Table 6 also showed that dry weight production of different varieties of jute seedlings
283 decreased significantly with increased level of Pb concentration. Possible reasons for such
284 declining trend might be due to the increased detrimental effect of Pb on growth of jute
285 seedlings.

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287 4.4.3 Effect of cadmium on fresh and dry weight

288 A significant response in respect of fresh and dry weight of different varieties of jute seedlings
289 was observed at 1% level of probability. Fresh and dry weight production decreased gradually
290 with increased level of Cd concentration.

291 4.4.4 Combined effect of arsenic, lead and cadmium on fresh and dry weight

292 Fresh and dry weight of different varieties of jute seedlings was significantly affected by
293 increased level of combined toxicity of As, Pb and Cd. Decreasing trend in terms of fresh and
294 dry weight of seedlings due to increased level of toxicity was observed in all 5 varieties.

295 Table 7. Effect of cadmium on fresh weight of different varieties of jute seeds

Varieties Treatments (mg Cd L ⁻¹)	Fresh weight (g)				
	Tosha pat o-9897	BINA desh pat-2	400 Gy P- 15(2)	500 Gy P- 65(2)	400 Gy P- 198(2)
0	2.8a	2.8a	2.5a	1.9a	2.0a
5	2.7a	2.7ab	2.1b	1.8a	1.9a
10	2.6a	2.5b	2.0bc	1.7a	1.7a
15	2.5ab	2.3c	1.8bcd	1.6ab	1.5ab
20	2.1b	2.1d	1.7c	1.3b	1.4b
30	1.8bc	1.7e	1.4d	1.0bc	1.0c
50	1.3c	1.4f	1.0e	0.9bcd	0.8c
70	0.8d	0.7g	0.8ef	0.5c	0.5cd
100	0.7de	0.6h	0.5f	0cd	0d
150	0e	0i	0g	0cd	0d
SE±	0.08	0.08	0.06	0.06	0.06
CV (%)	16.22	16.51	18.19	22.94	23.09
LSD	0.37	0.11	0.27	0.54	0.54

296 Note: P (≥0.01) means significant at 1% level of probability.

297 In a column figures with dissimilar letters differ significantly according to DMRT.

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301 Table 8. Combined effect of arsenic, lead and cadmium on fresh weight of different varieties
 302 of jute seeds

Varieties Treatments (mg As, Pb and Cd L ⁻¹)	Fresh weight (g)				
	Tosha pat o-9897	BINA desh pat-2	400 Gy P- 15(2)	500 Gy P- 65(2)	400 Gy P- 198(2)
0	2.5a	2.6a	1.9a	1.7a	1.9a
5	2.4ab	2.4a	1.8ab	1.6ab	1.8a
10	2.4ab	2.2a	1.7b	1.5b	1.7a
15	2.3b	2.1a	1.6bc	1.2c	1.6ab
20	2.2bc	2.0ab	1.1c	0.9d	1.1b
30	2.1bcd	1.8b	0.9d	0.7e	1.0b
50	2.0c	1.7bc	0.7e	0.65ef	0.9d
70	0.9d	1.0bcd	0f	0.6f	0.8bc
100	0.7de	0.7e	0f	0g	0c
150	0e	0d	0f	0g	0c
SE±	0.07	0.07	0.06	0.05	0.06
CV (%)	15.09	15.97	25.50	24.90	22.40
LSD	0.23	0.60	0.11	0.11	0.54

303 Note: P (≥ 0.01) means significant at 1% level of probability.

304 In a column figures with dissimilar letters differ significantly according to DMRT.

305

306 The experiment was conducted in the net house of the Department of Agricultural Chemistry,
 307 BAU, Mymensingh from October 2009 onwards to observe the growth performance of
 308 previously screened three jute varieties viz. BADC deshi pat (clv-1), BINA deshi pat-2 and Atom
 309 pat-38 in heavy metal contaminated soil. Results generated out of the second phase of the
 310 experiment revealed that, seeds of three varieties germinated in the toxic environment but their
 311 primary growth was not satisfactory. Reasons behind the stunted growth might be due to the
 312 unfavorable environmental...that prevailed during the growing season since off-season...trial
 313 with jute seeds were made to have jute in two seasons. We know bright sunshine coupled with
 314 rainfall is favorable for growth of jute plants but such environment was totally absent in the
 315 winter season.

316 Only primary growth of jute was studied in this phase of the experiment. Growth of different
 317 varieties of jute seedlings stunted severely in combined toxicity of heavy metal viz. As, Cd and
 318 Pb than single As contaminated soil. BINA deshi pat-2 though a photosensitive variety but in pot
 319 trial in off-season from October 2009 onwards failed to grow at minimum height not only at
 320 toxic condition but also under non-toxic soil as in control. If uptake of heavy metal by jute plants
 321 from contaminated soil is analyzed in the next season, then the heavy metal accumulating

322 potentiality of jute could be assessed. This income generating phytoremediation technology will
 323 be easily taken up by the farmers to eliminate heavy metal, build up in soil.

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325 **Summary and Conclusion**

326 Based on the results generated out of the first phase of the experiment following of jute seeds
 327 were made.

328 A. Grading of jute seeds against As, Pb and Cd toxicity

Sl. no.	Varietal class	Name of the jute variety
1.	Highly tolerant	BINA deshi pat-2
2.	Moderately tolerant	Tosha pat (0-9897)
3.	Tolerant	400 Gy P-15(2)
4.	Low tolerant	500 Gy P-65(2)
5.	Very low tolerant	400 Gy P-198(2)

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330 B. Grading of jute seeds based on varietal potentiality considering the results of control 331 treatment only

Sl. no.	Varietal class	Name of the jute variety
1.	Highly tolerant	BINA deshi pat-2
2.	Moderately tolerant	Tosha pat (0-9897)
3.	Tolerant	400 Gy P-15(2)
4.	Low tolerant	500 Gy P-65(2)
5.	Very low tolerant	400 Gy P-198(2)

332

333 The experimental findings of first phase revealed that BINA deshi pat-2 variety might have the
 334 potentially to serve in the toxic environmental conditions than the other four varieties. BINA
 335 deshi pat-2 variety was screened as the best variety to be used as a phytoremediant in the second
 336 phase of bioremediation process of removal of toxic heavy metal from contaminated soil.

337 Experimental findings of second phase of the experiment revealed that due to off season (from
 338 October 2009 onwards) pot trial seedlings of all the varieties failed to grow at minimum height
 339 not only at toxic environmental but also under non-toxic soil as in control. Unfavorable climatic
 340 conditions interrupted the primary growth of jute seedlings in pot culture. Only primary growth
 341 was studied in this phase. Heavy metal absorption by jute plants will be determined in the
 342 succeeding growing season. Metal accumulating efficiency of jute will be evaluated and jute will
 343 be recommended as phytoremediant for the removal of toxic heavy metals from contaminated
 344 soil.

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