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**Akanksha Raj**

Student, Department of Soil  
Science and Agricultural  
Chemistry, Bihar Agricultural  
University, Sabour, Bhagalpur,  
Bihar, India

**Jajati Mandal**

Assistant Professor-Cum-Jr.  
Scientist, Department of Soil  
Science and Agricultural  
Chemistry, Bihar Agricultural  
University, Sabour, Bhagalpur,  
Bihar, India

**Preety Bala Kumari**

Student, Department of Soil  
Science and Agricultural  
Chemistry, Bihar Agricultural  
University, Sabour, Bhagalpur,  
Bihar, India

**Corresponding Author:****Akanksha Raj**

Student, Department of Soil  
Science and Agricultural  
Chemistry, Bihar Agricultural  
University, Sabour, Bhagalpur,  
Bihar, India

## Organic amendment can reduce arsenic uptake in wheat

Akanksha Raj, Jajati Mandal and Preety Bala Kumari

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**Abstract**

A study was conducted to determine the efficacy of organic amendment in reducing arsenic uptake in wheat for this purpose soil was collected and recommended dose of N, P, K were added. The soil was spiked with five different level of arsenic (0, 10, 20, 30, 40 ppm). For organic amendment paddy husk and vermicompost were used, and the results revealed that the treatment with the paddy husk was found to be the best in comparison to vermicompost for amendment of arsenic. The arsenic accumulation in plants parts followed the order root>stem>leaf>economic produce and it was found that paddy husk was the better amendment for all the arsenic accumulated plant parts.

**Keywords:** Arsenic, organic amendment, paddy husk, vermicompost, economic produce

**Introduction**

Arsenic is a trace toxic element which is of great environmental concern due to its presence in soil, water, plant, animal and human continuum. Arsenic concentration as dissolved form in natural waters (except groundwater) are generally low, other than the areas which is characterized by the geothermal water or mining activities. The sedimentary rocks have higher arsenic content than that of igneous and metamorphic rocks. Out of 20 countries (covering Argentina, Chile, Finland, Hungary, Mexico, Nepal, Taiwan, Bangladesh, India and others) in different parts of the world where groundwater arsenic contamination and human suffering therefrom have been reported so far.

As small as 0.1g of arsenic trioxide can prove lethal to humans (Jarup, 1992) [8], due to its high toxicity and increased appearance in the biosphere has triggered public and political concern. The symptoms of chronic arsenic toxicity are dependent on the magnitude of dose and duration of its exposure. "Bell Ville Disease" in Argentina, "Black Foot Disease" in Taiwan and "Kai Dam" diseases in Thailand are well established as health disorder due to arsenic poisoning (Sanyal *et al.*, 2012) [20].

Since drinking water is considered as the most important source for Arsenic exposure, there are other sources also that leads to arsenic toxicity like soil-crop-food transfer. The world Health Organisation (WHO) recommended provisional guideline value of total arsenic concentration in drinking water is 10 µg As. L<sup>-1</sup>. Mehrag, (2004) [15] reported that accumulation of Arsenic in rice grain is considered as a calamity for South East Asia, where rice is grown as a staple food. Total arsenic content ranging from 10 to 20 ppm has found to be an index of arsenic hazard (Rahaman *et al.*, 2013) [18].

Various study is conducted which reveal that organic matter can be a effective source for reducing the concentration of arsenic in soil. As per findings of Mandal *et al.*, 2019 [12, 13] organic amendments reduced the As accumulation in wheat grain to the extent of 84% (sugarcane bagasse (SB)), 50% (rice straw) and 40% (paddy husk (PH)) compared with control. Sinha *et al.*, 2011 predicted that the organic manures added as soil amendment significantly reduced the accumulation (concentration) of arsenic in sesame seed to a maximum extent of 65.5% (vermicompost), 50% (phosphocompost), 42% (mustard cake) and 40% (farmyard manure (FYM)) compared with the control counterpart. Mandal *et al.*, 2019 [12, 13] reported that arsenic content in wheat grain was the lowest in sugarcane bagasse amended soil followed by farmyard manure (FYM) and vermicompost at all level of arsenic application. For this purpose a pot experiment was conducted to find the efficacy of organic matter used.

**Material and Method****Pot culture experiment**

The pot culture experiment was conducted to determine the As toxicity limits and symptoms in wheat (*Triticum aestivum*) plant. In each pot, 10 kg of the soil sample was placed with 5-6 seeds variety (HD-2733 taken from reputed seed company) sown in the pots.

The seeds were shown On 4 December, 2018. The experiment was laid out as a two factor Completely Randomised design (CRD) experiments with three replications and treatments were three levels of vermicompost at 10 t ha<sup>-1</sup> and rice husk given at 10 t ha<sup>-1</sup>. The recommended doses of NPK in the form of solution 150:60:40 kg ha<sup>-1</sup> were applied to the soils irrespective of treatment. The entire P and K fertilizers were applied basally, while N fertilizer was applied in two splits (50% as basal and the rest 50% top dressed at flowering).

### Preparation of soil and plant samples

The soil samples were air dried, ground and sieved through a 2 mm sieve and packed in airtight polythene containers. The plant samples were oven dried for 24 h at 105°C, ground and packed in airtight polythene containers. Soil samples were analysed for detailed characterization with respect to the important physiochemical properties. The standard doses of N,P,K fertilizers along with different levels of As (i.e., 0,10,20,30,40 ppm) pentavalent forms were applied to the pots at the time of sowing. The soils in the pot were irrigated with As free water and were maintained at the field capacity of the soil. Different yield attributes at different stages of crops, as uptake and accumulation parameters were measured.

Treatments details

Treatments	Arsenic doses + Organic sources
T <sub>1</sub>	0 ppm + Control (No amendment)
T <sub>2</sub>	0 ppm + Paddy Husk
T <sub>3</sub>	0 ppm + Vermicompost
T <sub>4</sub>	10 ppm + Control (No amendment)
T <sub>5</sub>	10 ppm + Paddy Husk
T <sub>6</sub>	10 ppm + Vermicompost
T <sub>7</sub>	20 ppm + Control (No amendment)
T <sub>8</sub>	20 ppm + Paddy Husk
T <sub>9</sub>	20 ppm + Vermicompost
T <sub>10</sub>	30 ppm + Control (No amendment)
T <sub>11</sub>	30 ppm + Paddy Husk
T <sub>12</sub>	30 ppm + Vermicompost
T <sub>13</sub>	40 ppm + Control (No amendment)
T <sub>14</sub>	40 ppm + Paddy Husk
T <sub>15</sub>	40 ppm + Vermicompost

### Experimentation

The pH of the soils and sludge were measured in 1:2.5 (Soil/Sludge: Water) suspension with the help of glass electrode digital pH meter. The electrical conductivity (Jackson, 1967) of filtrate of suspension was determined by electrical conductivity meter (elico® CM 180). Organic carbon of soil and sludge was estimated by chromic acid wet digestion followed by titrimetric measurement of unreacted dichromate (Walkley and Black, 1934) [27]. Cation Exchange Capacity determination involves saturation of the soil with an index cation (NH<sub>4</sub><sup>+</sup>), removal by washing of excess cation, and subsequent replacement of the absorbed index cation by another (Na<sup>+</sup>) and measurement of the index cation in final extract (Richards, 1954). The procedure of available nitrogen involves distilling the soil/ sludge with alkaline potassium permanganate solution and determining the ammonia liberated (Subbiah and Asija, 1956) [23]. Phosphorus was extracted by using spectrophotometer with Olsen reagent and ammonium acetate extractable potassium was determined by using flame photometer. Available micro-nutrients and heavy metal were extracted with the help of mixed solution of 0.005 M DTPA, 0.01 M Calcium Chloride and 0.1 M Triethanolamine (TEA) at pH 7.3 (Lindsey and Norvell, 1978). Total arsenic in plant samples, dried plant samples

(from pot culture) were digested on a sand bath with triacid mixture of trace element grade reagents (HNO<sub>3</sub>:H<sub>2</sub>SO<sub>4</sub>:HClO<sub>4</sub>:: 10:1:4, by volume) at 160°C for 4-5h, followed by the As measurement in (AAS-4141M) using external calibration of arsenate as standard.

### Instrumentation and analytical condition

Available arsenic determined by Olsen-extractable As using 0.5 molL<sup>-1</sup> NaHCO<sub>3</sub>. Instrumentation and analytical conditions. The leachate from soil or the digest of soil and plant sample was diluted to 50 mL. A 10 mL aliquot was placed in a 50 mL volumetric flask; 5 mL of concentrated HCl and 1 mL of mixed reagent (5% KI(w/v) + 5 % ascorbic acid (w/v) were added, left for 45 min to ensure complete reaction and the volume was made up to 50 mL. The resultant solution was analyzed in an atomic absorption spectrophotometer coupled with vapour generation accessory (VGA) at λ max = 193.7 nm, where the carrier solution was 10%(v/v) HCl and the reducing agent (to ensure all As species were reduced to and measured against a calibration with standard As<sup>+3</sup> solution in 10%(v/v) was 0.2% NaBH<sub>4</sub> in a 0.05% NaOH.

### Statistical analysis

Statistical analysis were performed using the INDOSTAT software (Indostat Services, Hyderabad, India).

### Results and Discussions

#### Characterization of the soils subjected to pot experiment

The physico-chemical properties of the initial soil samples is such that the pH is 8.2, electrical conductivity (EC) is 2.01 dSm<sup>-1</sup>, OC(%) is 0.47, CEC is 62.13 cmol (p+) Kg<sup>-1</sup>, available nitrogen determined is 165.13 Kg ha<sup>-1</sup>, available phosphorus is 17.21 Kg ha<sup>-1</sup>, available potassium is 224.56 Kg ha<sup>-1</sup> and available Fe, Mn, Zn, Cu in ppm are 51.75, 14.42, 4.57, 0.73 respectively.

#### Amendment of arsenic spiked soil

The As content in postharvest soil is shown in Table 1. The soil arsenic content was found to increase from 0 (control plot) to 2.42 ppm in 40 ppm As treated plot. The treatments were found to be statistically significant. Amendments with paddy Husk 10 tha<sup>-1</sup> proved to be the best causing considerable reduction in available arsenic with respect to control followed by vermicompost @ 10 t ha<sup>-1</sup>, 16% decrease in available arsenic was found with paddy husk amendments followed by 5% reduction with vermicompost with respect to control. An overall 12.5% decrease in available arsenic has been recorded against paddy husk with respect to vermicompost.

#### The interaction effect of graded doses of arsenic

Paddy husk amendments have been proved to be the best in reducing the soil available arsenic irrespective of the levels of As applied. This may be probably due to the binding capacity of the decomposition products of paddy husk like cellulose, hemicelluloses, lignin vis-à-vis enhancing the amounts of humic acid and fulvic acid and thus complexing with arsenic. The results are in accordance with the findings of (Mandal *et al.*, 2019 and Mandal *et al.*, 2019) [12, 13]. The soil organic fraction including HA and FA is known to combine with metal ions, clays, pesticides, and several organics because of high specific surface area and chelating abilities. Further, the humic substances also behave as good accumulators of toxic heavy metals, following the formation of metal-humate complexes (Chelates) with different degrees of stability

(Manunza *et al.*, 1995; Datta *et al.*, 2001) [14, 5]. The above mentioned theory of complexation also holds good with vermicompost which also enhances the organic matter as well as humic acid and fulvic acid. The organic manures added as soil amendments significantly reduced the available As content of soil compared with control counterpart as reported by Mandal *et al.*, 2019 [12, 13].

#### Accumulation of arsenic in grain per pot

Accumulation of As in grain per pot (ppm) is represented in Table-2. Accumulation of As increased with the increase in the graded dose of As from 0 ppm (control) to 18.21 ppm in 40 ppm dose. Application of amendments resulted in reduction of As accumulation high in paddy husk (17 % with respect to control) followed by vermicompost (6% with respect to control), depicts the interaction effect, which are found to be statistically significant. Amendments with paddy husk found to be effective in reducing As accumulation in grain with respect to other treatments irrespective of the levels of As. The reduction in the amount of available As in postharvest soil due to use of paddy husk and vermicompost as amendments resulted in the decline of accumulation of As in grain. Working with lathyrus + vermicompost+ poultry manure, Rahman *et al.*, (2011) [19] showed that combined application of all inputs reduced As transport in plant grain. The organic amendments reduced the As accumulation in wheat grain to the extent of 84% (sugarcane bagasse (SB)), 50% (rice straw) and 40% (paddy husk (PH)) compared with control (Mandal *et al.*, 2019) [12, 13].

#### Accumulation of arsenic in leaf

Application of graded doses of As increased the accumulation in leaf from 0 ppm in control to 27.26 ppm in 40 ppm dose as depicted in Table 3. However the intervention of amendments reduced the accumulation to the tune of 17.79% by paddy husk and 11% by vermicompost with respect to control. Interaction effects were also found to be significantly and paddy husk performed better than other treatments irrespective of levels of As in declining the As concentration in leaf.

#### Accumulation of arsenic in stem

Concentrations of As in stem of wheat crop is portrayed in Table 4. As concentrations ranged from 0 ppm (control) to 37.15 ppm (40 ppm As dose). A 20% decrease in As concentrations against amendments with paddy husk was found followed by 18 % with vermicompost with respect to control. Significant difference were observed in interaction effect of levels of As with amendments as observed from Figure 2. Amendments with paddy husk proved to be the best treatments irrespective of As doses.

#### Accumulation of arsenic in root per pot

Table 5 shows that arsenic accumulation in roots per pot. Highest concentration of 62.58 was observed with 40 ppm of as dose and the lowest 0 ppm with respect to control. Paddy husk reduced the accumulation by 15.555 with respect to control whereas vermicompost by 57%. Interaction effects were also found to be statistically significant as represented in Figure 1 and paddy husk has been proved to be the best amendment.

The As accumulation by the plant parts generally followed the order: root>stem>leaf>economic produce, in agreement with the findings of Das *et al.*, (2008) [3]. The As accumulation by plant parts was observed to increase with the increase of

added As. This was in agreement with the findings of Bhumbra & Keefer (1994) [2]. In this study greater arsenic accumulation was observed in wheat roots thus restricting the translocation to grain. Abedin *et al.*, (2002) [1] observed As accumulation in rice to be of the following order: root>stem>grain. For example, in pot trials in Bangladesh, Das *et al.*, (2004) [4] found 2.4 ppm As in rice roots, 0.73 ppm in stems and leaves and 0.14 ppm in grain. The values of our study were higher than the above values. This might be due to difference in level of available As in soil (Mandal 2012) [12, 13]. Irrespective of As treatments, roots contained higher concentrations of As than stem, leaf and grain in wheat reported by Zhang *et al.*, (2009) [30].

#### The dry matter weight of grain

The effect of different levels of As along with the soil amendments has been portrayed in Table 6 and Figure 4. A considerable reduction in grain dry matter weight was observed with the increase in the levels of As. The maximum dry matter weight of grain per pot of 24.01 g is recorded against control and maximum of 10.25 g against 40 ppm As. Addition of paddy husk As an amendments proved to be the best in increasing the dry matter weight of grain irrespective of the doses of As compared to vermicompost. 10.18% increase of grain dry matter weight per pot in paddy husk with respect to control treatments. And in vermicompost 8.73% grain dry matter weight is increased as compared no treatments. Whereas 1.31% increase of grain dry matter weight against paddy husk with respect to vermicompost.

The decrease in dry matter weight with increase in the level of As is due to the toxic effects of As. It may be attributed to the ability of arsenate which, by way of acting as a phosphate analogue is transported across the plasma membrane. This disrupts the energy flow in the cell, interferes in the oxidative metabolism and the photosynthetic mechanism, reacts with sulfhydryl groups (-SH) of enzymes and tissue proteins thereby reducing the accumulation of photosynthates within the sink (Ullrich-Eberius *et al.*, 1989) [25]. Intervention of organic manures (paddy husk and vermicompost) brought significant augmentation in dry matter yield by reducing the toxic effects of As doses. The addition of organic amendments to the soils can reduce heavy metals bioavailability by changing them from bio-available forms to fractions associated with organic matter. The probable cause for the observed decreases in exchangeable metal concentrations is the formation of complexes with particulate organics matter (Walker D.J. *et al.*, 2004) [26].

#### Relationship between arsenate and phosphate

The antagonistic relationship between arsenate and phosphate is well visualized from Figure 1,2,3 and 4. Increase in the As concentration in different plant parts resulted in decline of phosphate uptake. A series of negative correlation between As concentration and phosphate uptake is observed in case of root, stem, leaf, and economic produce.

Equation I: Root As content = -0.590 Leaf P content + 56.39 R <sup>2</sup> = 0.828
Equation II: Stem As content = -1.025 Stem P content + 47.24 R <sup>2</sup> = 0.751
Equation III: Leaf As content = -0.331 Leaf P content + 32.68 R <sup>2</sup> = 0.937
Equation:-IV: Grain As content = -0.493 Grain P content + 51.31 R <sup>2</sup> = 0.870

This may be attributed to the ability of arsenate which by acting as a phosphate analogue is transported across plasma membrane these findings are supported by the observations of (Meharg & Hartley-Whetaker 2001) [7] reported arsenate a chemical analogue of phosphate is taken up by *P. vittata*

transport system. Therefore it is expected that phosphate has competed with As for plant uptake. Wang *et al.*, (2002) [28] found that the membrane net arsenic influence in P starved plant increased by 2.5 times compared to control, while Tu *et al.*, (2004) [24] reported that the P addition to a hydroponic system reduced arsenic removal by *P. vittata*. Figure 5 more or less depicts the fact of chelation of arsenic by humic acid and fulvic acid and reducing its bioavailability having low  $R_2$  value (0.023). Increase in organic carbon content due to addition of amendments (paddy husk and vermicompost) compared to control have caused a declining trend of available As. Comparison with HA and FA fraction could have revealed a better picture. The negative correlation and the magnitude of such decrease in available As, however, varied with sources of organic matter, while such decrease remained most pronounced with paddy husk which might be due to formation of insoluble arseno-organic complexes and its adsorption on to organic colloids. These results are in concordance with the findings of (Sinha *et al.*, 2011) [21, 22] in case of sesame.

### Physicochemical properties of post-harvest soil

Table 7 depicts the physicochemical properties of post-harvest soil. The soil pH ranges from 7.97 to 8.10. In case of EC the lowest value recorded is 2.01 and the highest value is 2.09. Organic carbon content ranged from 0.45% to 0.61% where soil amendments were added. Minimum value of 63.29 (cmol (p+)  $\text{kg}^{-1}$ ) was recorded against CEC whereas maximum value observed was 65.55 (cmol (p+)  $\text{kg}^{-1}$ ). Available Nitrogen content of 148.93 kg/ha was recorded with respect to control whereas application of vermicompost increase it to 188.47 kg/ha. Phosphorus content ranged from 19.52 kg/ha against control treatment to 29.52 kg/ha with respect to paddy husk. In case Potassium the observed values ranged from 229.48 kg/ha to 255.78 kg/ha. Micronutrient Fe, Mn, Zn, Cu ranged from (51.08-52.17) ppm, (14.93-15.02) ppm, (4.93-4.95) ppm and (0.73-0.78) ppm respectively. All the parameters were found statistically non-significant with respect to the doses of arsenic as well as with the amendments added. However an increasing trend has been observed in all the parameters with respect to initial status but they were not statistically at per.

**Table 1:** Arsenic content in postharvest soils (ppm)

Arsenic levels	
0 ppm	0.00
10 ppm	0.42
20 ppm	0.91
30 ppm	1.72
40 ppm	2.42
S.Em(±)	0.003
CD(P=0.05)	0.008
Amendments	
No treatment	1.18
Paddy Husk @ 10t ha <sup>-1</sup>	0.98
Vermicompost @ 10t ha <sup>-1</sup>	1.12
S.Em(±)	0.002
CD(P=0.05)	0.006
Interaction effect	
S.Em(±)	0.005
CD(P=0.05)	0.014

**Table 2:** Arsenic accumulation in grain per pot (ppm)

Arsenic levels	
0 ppm	0.00
10 ppm	4.05
20 ppm	8.75
30 ppm	14.04
40 ppm	18.21
S.Em(±)	0.020
CD(P=0.05)	0.057
Amendments	
No treatment	9.77
Paddy Husk @ 10t ha <sup>-1</sup>	8.08
Vermicompost @ 10t ha <sup>-1</sup>	9.17
S.Em(±)	0.015
CD(P=0.05)	0.044
Interaction effect	
S.Em(±)	0.034
CD(P=0.05)	0.098

**Table 3:** Arsenic accumulation in leaf per pot (ppm)

Arsenic levels	
0 ppm	0.00
10 ppm	8.34
20 ppm	13.49
30 ppm	16.11
40 ppm	27.26
S.Em(±)	0.031
CD(P=0.05)	0.089
Amendments	
No treatment	14.44
Paddy Husk @ 10t ha <sup>-1</sup>	11.87
Vermicompost @ 10t ha <sup>-1</sup>	12.81
S.Em(±)	0.024
CD(P=0.05)	0.069
Interaction effect	
S.Em(±)	0.053
CD(P=0.05)	0.154

**Table 4:** Arsenic accumulation in stem per pot (ppm)

Arsenic levels	
0 ppm	0.00
10 ppm	9.49
20 ppm	21.71
30 ppm	28.52
40 ppm	37.15
S.Em(±)	0.030
CD(P=0.05)	0.088
Amendments	
No treatment	22.26
Paddy Husk @ 10t ha <sup>-1</sup>	17.79
Vermicompost @ 10t ha <sup>-1</sup>	18.08
S.Em(±)	0.023
CD(P=0.05)	0.068
Interaction effect	
S.Em(±)	0.052
CD(P=0.05)	0.152

**Table 5:** Arsenic accumulation in root per pot (ppm)

Arsenic levels	
0 ppm	0.00
10 ppm	14.72
20 ppm	32.12
30 ppm	41.32
40 ppm	62.58
S.Em(±)	0.077
CD(P=0.05)	0.224
Amendments	
No treatment	32.27
Paddy Husk @10t ha <sup>-1</sup>	27.75
Vermicompost @10t ha <sup>-1</sup>	30.43
S.Em(±)	0.060
CD(P=0.05)	0.173
Interaction effect	
S.Em(±)	0.134
CD(P=0.05)	0.388

**Table 6:** Grain dry-matter weight per pot (mg)

Arsenic levels	
0 ppm	24.01
10 ppm	21.40
20 ppm	16.99
30 ppm	14.93
40 ppm	10.25
S.Em(±)	0.027
CD(P=0.05)	0.080
Amendments	
No treatment	16.77
Paddy Husk @10t ha <sup>-1</sup>	19.22
Vermicompost @10t ha <sup>-1</sup>	16.55
S.Em(±)	0.021
CD(P=0.05)	0.062
Interaction effect	
S.Em(±)	0.048
CD(P=0.05)	0.138

**Table 7:** Physico-chemical status of post-harvest soils

Arsenic levels	pH	CEC (cmol (p+) kg <sup>-1</sup> )	EC (dSm <sup>-1</sup> )	OC (%)	N (Kg/ha)	P (Kg/ha)	K (Kg/ha)	Fe (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)
0 ppm	8.02	64.27	2.02	0.47	161.22	29.33	256.46	51.16	14.85	4.95	0.76
10 ppm	8.07	63.29	2.04	0.60	167.67	30.40	268.66	51.77	15.08	4.90	0.74
20 ppm	7.97	65.08	2.05	0.55	167.67	26.13	287.27	51.70	15.12	4.96	0.75
30 ppm	7.99	65.55	2.09	0.46	171.00	26.40	221.37	52.28	15.11	4.95	0.75
40 ppm	8.10	64.68	2.03	0.61	172.00	18.40	257.96	53.09	15.32	4.95	0.76
S.Em(±)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CD(P=0.05)	NS	NS0	NS	NS	NS	NS	NS	NS	NS	NS	NS
Amendments											
No treatment	8.06	64.16	2.05	0.45	148.93	19.52	229.48	52.02	14.93	4.95	0.73
Paddy Husk @10t ha <sup>-1</sup>	8.00	64.75	2.01	0.60	166.33	29.52	255.77	52.17	15.16	4.95	0.75
Vermicompost @10t ha <sup>-1</sup>	8.07	64.82	2.05	0.56	188.47	26.16	229.78	51.80	15.20	4.93	0.78
S.Em(±)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CD(P=0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Interaction effect											
S.Em(±)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CD(P=0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

## Conclusion

The above findings reveal the facts that amendment with the paddy husk helped in reducing arsenic concentration in comparison to vermicompost used in the treatment. A negative correlation was established between arsenic and phosphate uptake in root, stem, leaf and economic produce.

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