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Phytotoxicity Level and Effects of Arsenic Phytoextraction using Helianthus Annuus L. (Sunflower)

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Abstract
Arsenic is one of the most deadly contaminants polluting the environment in many countries of the world today. It occurs naturally in many ores (Copper, Lead, Gold etc.), but human activities (like explosions, mining, pesticides applications etc.) and natural occurrences (like volcanoes, micro-organisms activities) have increased its amount in the environment to lethal levels. This research involved the growing of sunflower plants *Helianthus annuus* L. collected from the Institute of Agricultural Research and Training (IAR&T) on various concentrations of Arsenate contaminated soil for Arsenic phyto-extraction for seven weeks to know the phyto-toxicity level of Arsenic on sunflower (an Arsenic hyper-accumulator). After several observations and statistical evaluations using the Analysis of Variance, it was discovered that as from 2.0g – 3.0g of Arsenate per kg of soil, 0% germination occurred. Between 0.75g – 1.5g of Arsenate per kg of soil, the percentage germination was 10% - 50% (not significant) and percentage survival was 30% (not significant). Furthermore, between 0g – 1.5g of Arsenate per kg of soil, there was a percentage germination of 60% - 100% (significant) and a percentage survival of 60% - 100% (significant). Hence, for efficient and appreciable Arsenic phyto-extraction from an Arsenate contaminated soil using Sunflower a concentration of 0.5g and below of Arsenate per kg of soil should be ensured. As from 0.75g of Arsenate per kg of soil (Phytotoxicity level) the effects of Arsenic phyto-toxicity observed are delayed germination, wilting, drying-off, damping-off, foliage chlorosis and necrosis, reddening etc.
Keywords: Phytoextraction, phytotoxicity, arsenic, sunflower, arsenate contaminated soil

1. Introduction

The sunflower plant *Helianthus annuus* L. is a native of America and the earliest known example of a fully domesticated sunflower was found at the Hayes site in Tennesse in the U.S and dated back to around 2300BC (Pope *et al.,* 2001). It is an annual plant and belongs to the family Asteraceae of the flowering plants. The plant has a large inflorescence "HEAD" and the plant can grow up to a height of about 3m, with the flower head reaching up to 30cm in diameter with large seeds. The head or inflorescence is a composite flower consisting of numerous florets (*www.wikipedia.com*, 2007). The sunflower plant requires full sunlight, fertile, moist, well drained soil with a lot of mulch for optimum growth. Sunflower plants are intolerant of acidic or water logged conditions (Pope *et al.,* 2001). As sunflowers have efficient root systems, they can be grown in areas which are too dry for many other crops. The fully or well established sunflower plants are drought resistant except during flowering (Duke *et al.,* 2001). Sunflower plant is used for many purposes such as yoghurt, bread, snacks, butter, livestock feed, cooking and industrial oil, hypoallergenic rubber, ornamental, phytoextraction of heavy metal contaminants from polluted environments etc. (*www.wikipedia.com*, 2007; Robt *et al.,* 1974),

The environment which is made up of the terrestrial, aquatic and atmosphere plays a major role to mankind as it serves as a habitat for every living organism, however man's activities has altered the balanced state of the earth, thus posing health hazards to life (Ramalingam, 2004). Through man's activities several pollutants of organic and inorganic origin have been released into the environment, where it has affected adversely the healthy state of man, animals and plants. In most cases death may occur which could lead to extinction as the biodiversity is destroyed (*www.wikipedia.com*, 2007).

Arsenic is a very toxic substance that is found in tiny amounts in some food items like sea foods, including products like cigarettes, laundry detergents, dolomite etc. (*www.essortment.com*, 2007). Arsenic contamination onto the soil is mainly through application of agricultural insecticides, copper smelting, mining, sheep dipping, chemical weapons, explosions and metallurgical industries (*www.essortment.com*, 2007). Volcanoes releases about 3,000 tonnes of Arsenic per year and microorganisms release volatile methylarsines of about 20,000 tonnes per year (*www.lenntech.com*, 2007). Once Arsenic is released into the environment, it cannot be destroyed therefore accumulating to toxic levels and giving rise to health effects in plants, animals and man, often resulting into death *(www.lenntech.com*, 2007). Exposure to Arsenic contaminants could be through skin contact, food, water or air, leading to dangerous effects like drowsiness, lungs irritation, cancer miscarriages, infertility, genetic distortion, cutting off of limbs, fingers and toes and death in man and animals (*www.lenntech.com*, 2007). The lethal dose of Arsenic is 763mg/kg by ingestion and 13mg/kg by intra-peritoneal infection. For a 70kg man this is about 53g (Saha, 2003)

Numerous measures have been taken by several scientists to control the problem of contamination. One of the steps is the phyto-remediation approach. Phyto-remediation is a concept which makes use of higher green plants to remediate a contaminated environment, while phyto-extraction is a technology under phyto-remediation which makes use of higher green plants to extract or remove contaminants from the environment (Kumar *et al.,* 1995).

Zhao *et al.,* (2002), used the Fern species *Pteris longifolia*, *P. umbrosia* and *P. cretica* to Phyto-extract Arsenic from contaminated soil. Wang *et al.,* (2001), used the Fern plant *P. vittata* to remove Arsenic from contaminated soil. Tassi *et al.,* (2004) used Lupine plants for Arsenic phyto-extraction. Also sunflower plants *Helianthus annuus* L. has successfully been used to phytoextract Arsenic (*www.wikipedia.com*, 2007).

Phyto-extraction has a lot of advantages compared to other remediation technologies because it makes use of plants which are easily monitored, poses minimal dangers, relatively cheap to establish, reduces the amount of waste on land, serves as breaks to wind and water erosion etc. (Belz, 1997). Despite all these merits governing phyto-extraction, it is still not optimally exploited in many countries because it is regarded as an upcoming remediation technology which requires a lot of research so as to understand every question asked about its efficiency.

Thus, the aims and objectives of this research is to determine the phyto-toxicity level of Arsenic compounds on the sunflower plant. This is because though the sunflower is an Arsenic hyper accumulating plant, but at a certain concentrations of Arsenic, the growth, development and survival of the plant in the contaminated soil is affected. Hence, the goal includes determining this level of phyto-toxicity and also the effects of the Arsenic contaminants on the plant.

3. Materials and Methods

Data was collected on the experimental set-up for the plant height, leaf area, number of leaves, girth, percentage germination, percentage survival. After day 1 of planting the data was collected for 7 weeks. The morphology was observed for symptoms of Arsenic poisonings. The plant height and leaf area was measured with the aid of a meter rule; the number of leaves was counted, while the Girth was measured with the aid of calipers. The percentage germination was determined by counting the number of seeds that germinated per treatment. Also, the percentage survival was determined by counting the number of plants that survived the whole 7 weeks of research. In a treatment, there were 2 replicates and 5 seeds were planted in each replicate.

Hence:-

Percentage germination or Percentage survival =

$$\frac{\text{Percentage germination or survival}}{10} \times 100$$

After which the Analysis of Variance (ANOVA) and Least Significant Difference were used to test the significance of the results.

4. Results and Discussion

Table 1. The morphological observations, percentage germination and percentage survival for Block C Evening sun cultivar.

		Week 1	Week 2	Week 3	Week 4	Percentage Germination (%)	Percentage Survival
Row 4	EsAs	1 seed germinated	1 seed germinated but developed pre-emergence damping off.	1 seedling developed shoot blight and died.	-	40	0
	EsAsP	2 seeds germinated	1 seedling developed stem rot and died.	1 seedling developed foliage wilting and drying.	1 seedling developed stem rot and died.	40	0
	EsAsS	3 seeds germinated	2 seedlings developed stem rot and died.	1 seedling developed stem rot with shoot reddening.	1 seedling showed leaves necrosis, dried up and died.	60	0
	EsCntrl	3 seeds germinated	-	2 seeds germinated	-	100	100
Row 3	EsAs	2 seeds germinated	1 seed germinated	-	2 seedling showed foliage necrosis and chlorosis then died	60	20
	EsAsP	-	2 seeds germinated.	1 seed germinated	1 seedling died.	50	30
	EsAsS	4 seeds germinated	-	-	-	80	80
	EsCntrl	4 seeds germinated	-	-	-	80	80
Row 2	EsAs	4 seeds germinated	-	-	-	80	80
	EsAsP	2 seeds germinated	1 seed germinated	-	-	60	60
	EsAsS	4 seeds germinated	-	-	-	80	80
	EsCntrl	5 seeds germinated	-	-	-	100	100
Row 1	EsAs	5 seeds germinated	-	-	-	100	100
	EsAsP	4 seeds germinated	-	-	-	80	80
	EsAsS	5 seeds germinated	-	-	-	100	100
	EsCntrl	3 seeds germinated	2 seeds germinated	-	-	100	100

Es= Evening sun cultivar, **As**=Arsenate alone added to soil, **AsP**=Arsenate with Phosphate added to soil, **AsS**=Arsenate with Sulphate added to soil.

Table 2. The morphological observations, percentage germination and percentage survival for Block B Evening sun cultivar.

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Percentage germination (%)	Percentage survival (%)
Treatment 4 (T_4)	-	-	-	-	-	-	-	0	0
Treatment 3 (T_3)	-	-	-	-	-	-	-	0	0
Treatment 2(T_2)	-	1 seed germinated	1 seedling rotted and died	-	-	-	-	10	0
Treatment 1(T_1)	-	-	2 seeds germinated	1 seed developed stem rot, then the plant collapsed and died.	1 seed germinated	1 seedling developed damping off and died	-	30	10

From table 1 and 2, it shows that between 0.0g – 0.5g of Arsenate per kg of soil the percentage germination was 60% - 100% while the percentage survival was also 60% - 100%. Then between 0.75g – 1.5g of Arsenate per kg of soil the percentage germination was 10% - 50% while the percentage survival was 30%. From 2.0g – 3.0g of Arsenate per kg of soil the percentage germination is 0.0%.

Two Tail ANOVA (P=0.05) for Phytotoxicity level

Table 3. The Analysis of Variance (ANOVA) for Percentage germination of Sunflower between Arsenate concentrations of 0.0g to 0.5g per kg of soil.

	Replicate 1	**Replicate 2**	**Source of Variation**	**Degree of freedom**	**Sum of Squares**	**Mean of Squares**	**F- Value**
Treatment 3	4	2	Treatment	2	4	2	a. (Calculated value) 19.00 (Tabulated value).
Treatment 2	3	5	Replicate	1	0	0	
Treatment 1	5	5	Error	2	4	2	
			Total	5	8	1.6	

The percentage germination is significant because the calculated F – value is lower than the tabulated F – value.

Table 2 and 3 showed that both the percentage germination and survival of the sunflower plants between the Arsenate concentrations of 0.0g to 0.5g per kg of soil are significant since their calculated F – values are lower than their corresponding tabulated F – values. Thus, the Least Significance Difference LSD is calculated to know if the difference between two consecutive treatments is significant.

$LSD = t_{0.05} \sqrt{(2MSE/R)}$

(LSD = Least Significance Difference, MSE = Mean Square value for Error, R = number of Replicates).

LSD = 6.08

NOTE: The difference between the Treatment 0.25 is smaller than the LSD, hence there is no significant difference the Treatments for Percentage germination of the Sunflower plants grown on soil of concentration between 0.0g to 0.5g.

Also, by using the LSD it shows that the LSD for Percentage survival is also 0.25. hence, there is no significant difference between the treatments for Percentage survival of Sunflower plants grown on soil concentration between 0.0g to 0.5g.

Table 4. The Analysis of Variance (ANOVA) for Percentage germination of Sunflower between Arsenate concentrations of 0.75g to 3.0g per kg of soil (above).

	Replicate 1	Replicate 2	Source of Variation	Degree of freedom	Sum of Squares	Mean of Squares	F - value
Treatment 5	0	0	Treatment	4	11	2.75	7.86 (Calculated value)
Treatment 4	0	0	Replicate	1	1.6	1.6	6.39 (Tabulated value).
Treatment 3	1	0	Error	4	1.4	0.35	
Treatment 2	3	1	Total	9	14	1.56	
Treatment 1	3	2					

The percentage germination is not significant because the calculated F – value is higher than the tabulated F – value.

Table 5. The Analysis of Variance (ANOVA) for Percentage survival of Sunflower between Arsenate concentrations of 0.75g to 3.0g per kg of soil.

	Replicate 1	Replicate 2	Source of Variation	Degree of freedom	Sum of Squares	Mean of Squares	F - value
Treatment 5	0	0	Treatment	4	3.6	0.9	9.00 (Calculated value)
Treatment 4	0	0	Replicate	1	0.1	0.1	6.39 (Tabulated value).
Treatment 3	0	0	Error	4	0.4	0.1	
Treatment 2	0	0	Total	9	4.1	0.46	
Treatment 1	2	1					

The percentage survival is not significant because the calculated F – value is higher than the tabulated F – value.

Table 4 and 5 showed that the percentage germination and survival between concentrations of 0.0g – 0.5g of Arsenate per kg of soil are both not significant because their calculated F – values is higher than their tabulated F – values.

The reason why the Sunflower plants had a significant percentage germination and survival between 0.0g – 0.5g of Arsenate per kg of soil, was because these concentrations were still tolerable by the seeds for germination and survival and also to phytoextract the Arsenic. While between 0.75g – 3.0g the percentage germination and survival of the Sunflower plants were not significant because the concentration of the Arsenate was too high to support germination, survival and phytoextraction. According to Kabata-Pendias and Pendias, (1992), many non-arsenic hyper accumulating plants have a phytotoxicity level of between 0.005g to 0.1g of Arsenate per kg of soil.

As from 0.75g of Arsenate per kg of soil phytotoxicity symptoms started to appear on the Sunflower plants used for Arsenic phytoextraction from the Arsenate contaminated soil. The symptoms observed include (in no particular order): wilting, stem reddening, stem bending, leaf crinkling, pre-emergence and post-emergence damping off, delayed germination and stunting of plants, leaf holes. Wang *et al.,* (2001), suggested that phytotoxic symptoms during Arsenic toxicity in plants include necrosis and chlorosis of leaves, poor growth, delayed germination etc.

In conclusion, for efficient and appreciable Arsenic phytoextraction from an Arsenate contaminated soil using sunflower plants a concentration of 0.5g and below of Arsenic per kg of soil should be ensured. Also, when using sunflower to phytoextract Arsenic from contaminated soil where the concentration is above 0.75g per kg of soil effects of symptoms of Arsenic toxicity will begin to appear on the plants.

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