ATIPC – 2020 Special Issue

J. Indian Chem. Soc.,
Vol. 97, No. 12b, December 2020, pp. 2758-2764

Impact of soil addendum on arsenic uptake by rice plant in the alluvial soil of gangetic West Bengal, India

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Manuscript received online 06 December 2020, accepted 26 December 2020

The present work is a comparative research between application of soluble and insoluble chelator as soil addendum against arsenic (primarily As^{3+}) toxicity on rice plant (*Oriza sativa* L.) in Gangetic alluvial soil. Experimental results showed that soil arsenic (As) content decreased (0.15 to 0.08 mg/kg) distinctly by using soluble chelator of disodium ethylenediamine tetra-acetic acid (DSEDTA) due to phytoremediation through plant uptake. This was further authenticated by the higher level (2.6 to 2.88 micro-mole/g) of the stress marker compound (malonaldehyde) produced in the plants to combat the stress due to As accumulation. Plant stress, in the present study was also supported indirectly with the help of other biochemical parameters like chlorophyll, carbohydrate and protein. It was observed that the malonaldehyde contents in the plants assisted by DSEDTA soil have quite higher compared to the plant of EDTA assisted soil (reduction from 2.69 to 1.9 micro-mole/g, for 1 g/kg of chelator). The plants cultivated in EDTA assisted soil, plant health indicators like height of the plant, leaf chlorophyll, carbohydrate and protein content were recorded comparatively higher. The probable reason behind this fact could be hypothesized that the insoluble chelator (EDTA) might play some protective role that may be responsible to avoid plant uptake of As from the soil solution.

Keywords: Soil arsenic, plant uptake, stress markers, insoluble and soluble chelator, rice plant.

Introduction

Arsenic (As) is a toxic metalloid and ubiquitous in the environment. Rising levels of As in soil is primarily due to anthropogenic inputs. It has become a major threat to living organisms including human being due to the adverse consequences. United States (US) Environmental Protection Agency (EPA) identified arsenic as a powerful human cancer-causing agent and a main source of diseases of the skin, lung, bladder, liver, and kidney in the human system[1,2].

As is a redox-sensitive metalloid, arsenate [As^{5+}] and arsenite [As^{3+}] are commonly found in soil. The two inorganic As species are readily interconvertible depending on the environmental conditions. Arsenate [As^{5+}] predominates under aerobic conditions, whereas anaerobic conditions in flooded paddy soil favor arsenite [As^{3+}][3]. A range of factors like, physico-chemical, biological and environmental govern the speciation and mobility of As in paddy soil-water systems[4].

One of the major reasons for increase in soil As is due to irrigation with As contaminated water. Rice being a major cereal in West Bengal (India) there is a fair reason for bioaccumulation of As among rice eating people of Bengal. A study was conducted to assess the potential risk of skin lesions within the children due to consumption of arsenic-contaminated rice in West Bengal, shows more adverse effect on the children of 16–18 compared to lower age group as rice is the staple food for this higher age range[5].

Paddy plant (rice) is more susceptible to As accumulation than other cereals because of the high mobility of arsenic under the flooded condition in the rice field[6]. The soil environment in paddy cultivation is reducing and out of two oxidation states of arsenic the arsenite (As^{3+}) form which is 60 times more soluble, toxic and mobile than arsenate (As^{5+}) is mostly found in this reducing soil condition[7]. Hence to ensure fewer uptake by the plants becomes the prime concern to address this problem.

The use of plants for cleaning up the contaminated soil has gained scientific credibility for last few decades. The main emphasis has been to use the plants with natural ability to hyperaccumulate the toxicants. Several chemical reagents particularly chelating agents are tried in agronomy for analysing the soil trace metals. Among the chelating agents, DSEDTA increases the bioavailability of the heavy metals and plant uptake from the soil due to its strong chelating ability. Sodium salt of EDTA application promotes the solubility of heavy metals up to 80% and makes it available for phytoremediation[8,9].

Phytoremediation offers a low cost eco-friendly solution to soil contaminants though it has got some limitations. This has little impact in case of large area decontamination especially in low concentration of contaminants it does not seem to be feasible. Plant growth depends on a number of environmental parameters and even under best possible environmental conditions the removal by plants is limited. In such case the phytoremediation might be followed by some other remediation mechanism which may have some economic implications[10]. Also phytoremediation is restricted for the contaminants which are non-hydrophobic as the basic principle is the solubility of the contaminant. In DSEDTA assisted phytoremediation, arsenic taken up by the plants is mostly in tri-valent form (As^{3+}), which is more toxic. Thus the biomass generated by phytoremediation is highly toxic and it needs proper disposal to avoid further soil contamination. So, to make the phytoremediation process a complete one there has to be a strong waste management policy associated with it.

In the light of the above facts, the objectives of this present work are to draw a comparison between application of soluble (DSEDTA) and insoluble chelator (EDTA) as soil addendum in arsenic uptake on rice plant (*Oriza sativa* L.) in Gangetic alluvial soil; also to highlight the pattern of variation of some bio-chemical components as well as the degree of stress imparted due to As accumulation in the plant; and to explore the possibility of an alternative way to grow the plants in moderately arsenic contaminated soil with insignificant level of accumulation.

Experimental set up

Rice is the staple grain and is the maximum consumed form of carbohydrate in West Bengal. It serves as one of the easiest ways to human contamination of As through daily uptake. Rice is a monocot plant, belongs to the Gramineae family and the genera of Oryza. Rice variety *Shatabdi* (IET4786) was used for the experiment. The set-up was done in September 2019 and harvesting was during January 2020.

The rice seed was collected from Rice Research Station, Chinsurah, West Bengal and allowed to germinate. 7–10 days old seedlings were first sown in few random pots. The plants were then transplanted after 15 days to 18 different pots, 6 experimental in triplicate set (Table 1). The average of the three observations was considered. Each pot was prepared with 4 kg soil and five rice saplings. The addendums, EDTA or DSEDTA and arsenic were mixed according to the doses mentioned (Table 1), EDTA salt was added in the ratio of 1 g/kg, 2 g/kg and 3 g/kg in the soil[11]. To allow the normal growth of the plant 50 g of compost fertilizer was added in each pot. All the pots were randomly placed in the university nursery to ensure that each plant gets similar exposure on available sunlight, air flow, temperature fluctuations and other environmental factors. Plants were irrigated frequently with tap water and the water which was leached out of the pot was also reintroduced back into the pot.

Table 1. Experimental set-up showing pot marking and the combination of addendums used during the experiment

1.	Experimental 1 with EDTA	1 g/kg EDTA + 30 mg/kg arsenic + 4 kg soil
2.	Experimental 2 with EDTA	2 g/kg EDTA + 30 mg/kg arsenic + 4 kg soil
3.	Experimental 3 with EDTA	3 g/kg EDTA + 30 mg/kg arsenic + 4 kg soil
4.	Experimental 4 with disodium-EDTA	1 g/kg DSEDTA + 30 mg/kg arsenic + 4 kg soil
5.	Experimental 5 with disodium EDTA	2 g/kg DSEDTA + 30 mg/kg arsenic + 4 kg soil
6.	Experimental 6 with disodium EDTA	3 g/kg DSEDTA + 30 mg/kg arsenic + 4 kg soil

Method of estimation:

Estimation of soil parameters:

The pH of the composite soil sample was measured in the ratio of soil: solution of 1:2.5. The samples were equilibrated to the normal temperature and pH of the soil was

measured by digital pH meter (Model No. Systronics-802). After the plants were grown and harvested the soil sample was collected from each pot by composite sampling. The samples were stored in zip lock bags and soil As concentration was estimated in HCl digested solution by Atomic Absorption Spectrophotometer (AAS NOVA 350 model equipped with HM 55, German made).

Estimation of bio-chemical parameters:

For estimation of malondialdehyde content, the sample extract was prepared from fresh plant by grinding, centrifuging and collecting the supernatant. To the supernatant, 20% TCA (trichloroacetic acid) and 0.5% TBA (thiobarbituric acid) were added and mixed well. The mixture was boiled and then quickly cooled on ice. The mixture was then centrifuged. The supernatant was collected and the absorbance was recorded at 532 nm by Digital Photo Colorimeter, Model No. LT-12, LABTRONICS. The concentrations were calculated on comparing with the standard curve[12].

For total chlorophyll content, the fresh plant samples were ground and extracted in acetone and supernatant was collected for preparation of the sample extract. This procedure is repeated till the residue is colorless. The absorbance was measured at 645 nm, 663 nm, 652 nm in Digital Photo Colorimeter and chlorophyll concentration was calculated by standard formula as outlined[13] in the protocol.

Total carbohydrate in plant was estimated by employing standard method. The samples were acid digested and then neutralized with sodium carbonate, centrifuged, supernatant collected. To it, anthrone was added, boiled, cooled and the absorbance recorded at 630 nm by Digital Photo Colorimeter. The concentrations were found out by graphical plotting against a standard curve[13].

For total protein, the fresh plant samples were ground, centrifuged and supernatant collected for preparation of the sample extract. To the extract alkaline copper solution was added and mixed well followed by addition of Folin- Ciocalteau reagent, mixed well and incubated in the dark for 30 min. The absorbance was measured at 660 nm by Digital Photo Colorimeter. The concentrations were calculated against a standard curve[13].

Statistical analysis:

All the analytical data have been compared by statistical analysis. Pair tests were performed to find the correlation coefficient with p value. The results of p value within 95% confidance level were considered. All the statistical analysis have been done using Microsoft excel.

Results and discussion

Soil arsenic concentration:

The pH of the soil varied from 6.4 to 6.6 and was always less than the neutral level of 7.0, probably due to presence of anaerobic decomposition products of soil organic matter. The texture of soil was visually observed to be of silty-clay in nature, indicating occurrence of large fractions of finer particles which could increase the water holding capacity and could show higher extent of absorption-desorption capability with the metal ions. Hence, addition of any chelating agent might have to compete for the metal ions with soil exchange capacity. EDTA being a popular chelating agent has been used in removing heavy metals from soil due to its high chelating ability[14]. The present study revealed (Fig. 1) that in the DSEDTA treated plants, after harvesting the soil As content was reduced much compared to EDTA assisted soil. The reduction is highly statistically significant with the dose of 3 g

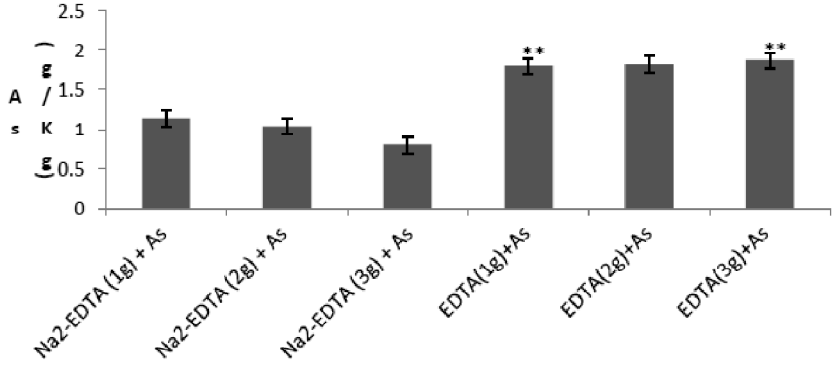

Fig. 1. Soil As content shows variation due to use of different chelators.

of DSEDTA ($p < 0.01$). This evidently corroborated the fact that soil As reduction could be due to plant uptake keeping other environmental and soil factors constant. On the other hand, EDTA assisted soil As was not taken up by the plants to that extent and the soil As content was not reduced much. The reduction of soil As in DSEDTA assisted soil was possibly due to formation of strong and soluble metal-chelate complex in the rhizospheric soil which is case of EDTA application is insoluble and did not allow the metal to reach to the plant root surface in available form.

Variations among different bio-chemical plant stress markers:

It has been known that As interacts within the biological system via two routes i.e. by replacement of mandatory ions from the active sites of the proteins, or by direct inactivation of the key enzymes, either through interaction with sulfhydryl groups or indirectly due to generation of ROS (Reactive Oxygen Species), thus resulting in a cascade of irreversible injuries in plants[15]. As-induced stress can provoke several toxic effects at cellular and molecular level in the plants. To cope up with the situation of arsenic toxicity, plants develop various tolerance mechanisms. Those lead to several biochemical and physiological changes which affect metal uptake and root-shoot transport of metal ions by the plants[16].

In rice plant high level of As exposure reduces root and shoot length, chlorophyll and both essential and non-essential amino acids in plant and food grains have been reported by many researchers[17,18]. In the present study, it was observed that in EDTA assisted plants, the contents of carbohydrates varied from 0.49 to 0.59 mg/g (Fig. 3), chlorophyll content from 2.25 to 2.41 mg/g, and protein content ranged from 0.18 to 0.2 mg/g (Fig. 2 and Fig. 4). On the other hand,

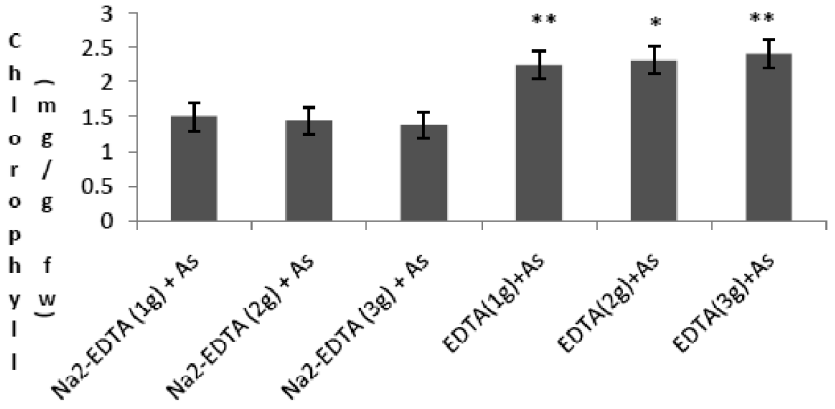

Fig. 2. Chlorophyll level in plants cultivated with differential treatment.

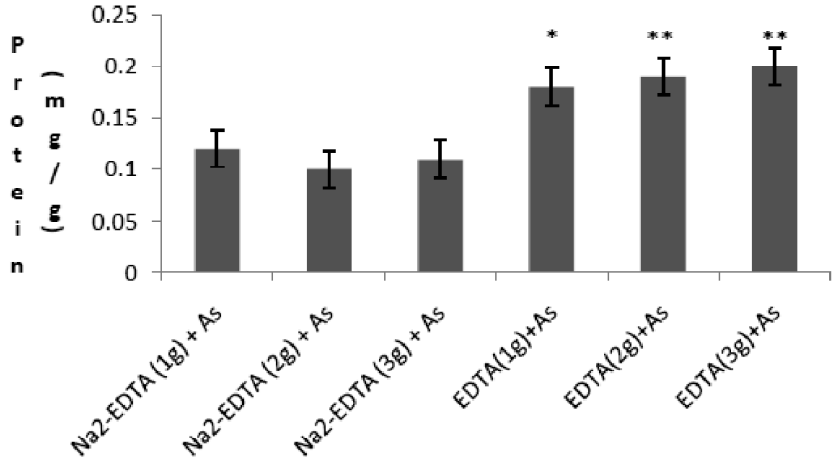

Fig. 3. Protein content in plants cultivated in soil treated with different chelators.

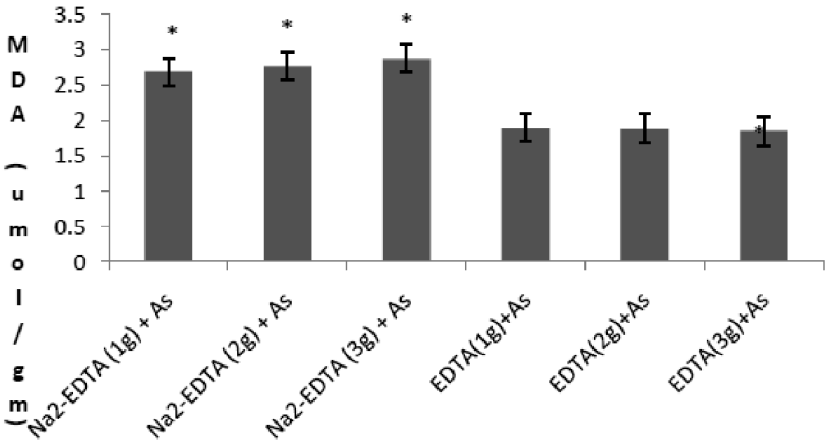

Fig. 4. MDA content shows variation among the cultivated plants.

in DSEDTA assisted soil, carbohydrate values were between 0.33 and 0.26 mg/g, chlorophyll ranged within 1.5 to 1.38 mg/g and protein in the range between 0.12 to 0.11 mg/g. These differences could be ascribed due to difference in As generated toxicity in the plants due to two types of chelator treatment.

Variations of oxidative stress markers:

Malonaldehyde (MDA) content is a general marker of oxidative stress in a cell due to increased lipid peroxidation[19]. In the present work, it was evident that due to higher As uptake by the plants treated with DSEDTA, the level of lipid peroxidation increased and were reflected in proportionate increase in MDA content almost 1.5-folds than the plants raised in EDTA treated soil (Fig. 4). In the present work the significant decrease in protein content in the plants raised in DSEDTA treated soil compared to EDTA treated soil could be directly correlated with the As induced oxidative stress on plants.

Comparison of stress on plant height during a month of cultivation:

Lower content of chlorophyll, carbohydrate and protein in DSEDTA assisted soil must have played a negative role in plant growth which could be measured by plant height. Studies on plant growth also highlighted (Fig. 6) that upto day-5, there were no significant changes in the plant height in both types of treated plants. However, major differences were

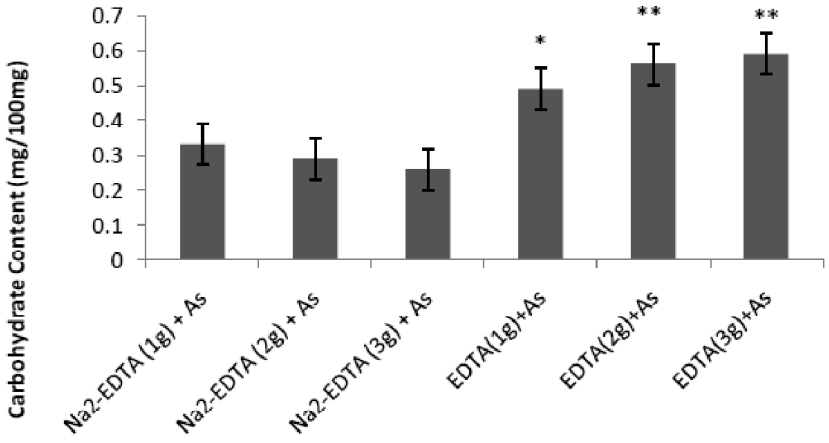

Fig. 5. Carbohydrate content in the plants cultivated with EDTA and DSEDTA.

observed from day-15 during the first one month of the experiment. It was evident from the Fig. 6, that the maximum plant growth were recorded in EDTA assisted soil and on an average varied from minimum of 11.8 cm at day-15 to the maximum of 24.9 cm at day-30 with 3 g/kg dose of chelator. Compaing with the plant height between DSEDTA and EDTA treated soil, it was predicted that the difference in plant heights were directly related to the days of exposure along with the chelator type. The more the duration of As exposure, the

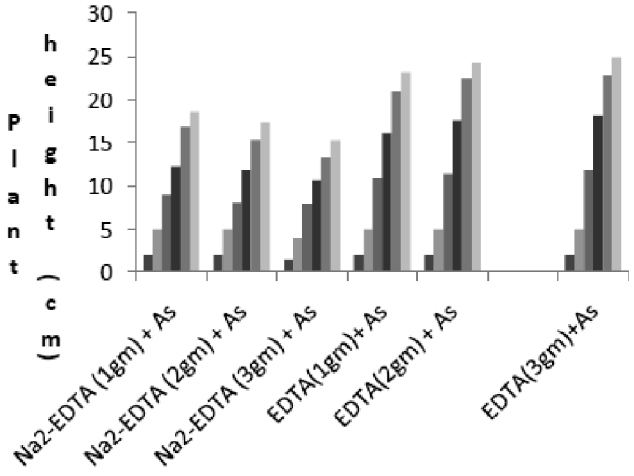

Fig. 6. Plant height in cm cultivated in soil treated with different chelators.

more were the differences in the plant height as registered during 20 to 30 days. This experimental results well substantiate the earlier research that arsenic interferes with metabolic processes and develops arsenic-induced phytotoxicity which leads to substantial reduction in chlorophyll, carbohydrate and protein content and might have negative impact on the rate of photosynthesis of the plant which in turn, inhibit the plant growth[20].

Conclusions

The objectives of this study was to understand the difference in the behaviour of both types of chelators (DSEDTA and EDTA) regarding As uptake by the rice plant. The study clearly indicated that the plants grown in the soil treated with DSEDTA had the preference to uptake more arsenic from the soil solution compared to the plants cultivated in the EDTA treated soil which was reflected both by the lower level of soil As content and the plant stress markers. Soil As contents in DSEDTA treated soil were much less compared to EDTA treated soil. The plant stress marker MDA content also substantiated the same fact. From the experimental data analysis, it could be hypothesized that EDTA application can save the plant from As related toxicity to some extent. However, it must be mentioned that this process is not helping in soil remediation from As but it could only save the plant as well as the consumers of the crops from the arsenic toxicity. Phytoremediation though an eco-friendly method but it must include an efficient mechanism of waste management system, but this is not a concern for an insoluble chelator. Further work is required in this to understand the right dose of chelator application to get the best result.

Acknowledgements

The authors are indebted to the management of University of Engineering & Management Kolkata for the financial support.

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