International Journal of Agriculture, Environment and Biotechnology
Citation: IJAEB: 11(1): 45-54, February 2018
DOI: 10.30954/0974-1712.2018.00178.6

ENVIRONMENTAL SCIENCE

Vigna radiata: A Potent Phytofiltrator of Lead Grown Hydroponically in Sewage

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Paper No. 649 Received: 14-09-2017 Accepted: 18-01-2018

ABSTRACTS

Phytofiltration is an ecofriendly technique, which use plants biomass to remove toxic heavy metals from aqueous waste. Lead (Pb) present in sewage induces oxidative stress and cause deleterious effects on living organism, hence it has to be removed. The aim of the study is to evaluate efficiency of *Vigna radiata* as phytofiltrator of lead. The efficiency was estimated in terms of growth attributes, stress marker and accumulated lead content of *V. radiata*. In the present experimental design, *Vigna radiata* (Mungbean) plants were grown hydroponically in sewage and 50% diluted sewage for 15-days. *V. radiata* grown in Hoagland media served as the control. Accumulated lead content was estimated by Atomic absorption spectroscopy. It was observed that total height, fresh and dry weight of *V. radiata* was not changed significantly ($p>0.05$) in sewage as compared to control. Reduction in total height was significant ($p<0.05$) and fresh and dry weight was highly significant ($p<0.01$) in 50% sewage. Lead accumulation was observed in *V. radiata* grown using sewage. Malondialdehyde (MDA), peroxidase, and proline content increased significantly ($p<0.05$) in sewage when compared to control. In 50% diluted sewage, malondialdehyde, peroxidase reduced significantly ($p<0.05$), while proline content showed a significant increase ($p<0.01$) when compared to undiluted sewage. It was concluded that *V. radiata* has potential to phytofilter lead from sewage. Increased MDA level indicates oxidative stress. Increased antioxidative stress markers; proline and peroxidase activity shows that *V. radiata* has tolerance to lead stress. Use of 50% diluted sewage is effective in reducing oxidative stress but it also negatively affects plant growth when compared to undiluted sewage.

Highlights

- *Vigna radiata* has potential to phytofilter lead from sewage.
- Increased peroxidase and proline content indicates that *V. radiata* has efficient detoxification mechanism to tolerate lead stress.

Keywords: Growth attributes, Malondialdehyde, Peroxidase, and Proline.

Hydroponic is defined as a science of growing plant in soil less artificially prepared nutrient media. Static solution culture is a type of hydroponic technique in which plants are grown in static reservoirs of nutrient solution (Reshma and Sarath, 2017). Phytofiltration is a subset technique of phytoremediation, which use hydroponically grown plants to filter toxic metals from aqueous waste (Kumar *et al*. 2017). In this technique, specifically engineered plants were used to clean up hazardous pollutants from the aqueous solution by detoxifying, extracting and hyper accumulating it in tissues (Tangahu *et al*. 2011). Phytofiltration process is of three types: a) Rhizofiltration: plant roots, b) Blastofiltration: seedlings, c) Caulofiltration: excised plant shoots (Razzaq, 2017). Some plant species have the potential to absorb and accumulate toxic metals in root or shoot and biodegrade or biotransform them into inert form (Nguyen *et al*. 2016). Such plants also have efficient metal detoxification mechanism to tolerate stress conditions (Kumar *et al*. 2013). Indian mustard has a bioaccumulation coefficient of 563 for lead and has also proven to be very effective in removing a wide concentration range of lead (4 mg/L -500 mg/L) (Raskin and Ensley 2000). Terrestrial plants are generally more preferred when compared to aquatic plants because they have a fibrous and much longer root system to accumulate more amounts of metals (Dushenkov *et al.* 1995). The integration of phytofiltration with waste water treatment has been proposed as an ecological alternative where ex-situ and in-situ removal of toxic metal from aqueous waste is possible by growing metal accumulator plants hydroponically in it.

Lead is one of the toxic heavy metal which gets dumped into fresh water bodies through various sources like storage battery industries, lead paints factories, mines and smelters of lead ores (Pinho and Ladeiro 2012). Such water is used commonly to irrigate agricultural fields. Plants absorb lead from soil via apoplastic pathway or Ca^{2+} permeable channels and accumulate it in root cells (Pourrut *et al*. 2011). Lead (Toxic heavy metal) is ranked first as a heavy metal pollutant and second of all hazardous substances (ATSDR 2007). It enhances generation of reactive oxygen species (ROS) like hydrogen peroxide (H_2O_2) via Fenton and Haber-Weiss reaction and induces oxidative stress in plants. ROS cause oxidative deterioration of polyunsaturated fatty acid of lipid membrane into malondialdehyde and changes intrinsic properties of the membrane (Ayala 2014). High lipid peroxidation overwhelms repairing mechanism and induces apoptosis (Beltagi and Mohamed 2013). Lead also enters in the food chain and cause carcinogenic and mutagenic effect on human beings (Rahoui *et al*. 2008).

Antioxidative defence mechanism consists of enzymatic and non-enzymatic antioxidant molecules. Peroxidase is heme protein enzyme, which reduces hydrogen peroxide (H_2O_2) into water and molecular oxygen (Aki *et al*. 2009). Proline is a key osmolytic amino acid. It acts as a signaling molecule which modulates mitochondrial functions, influence cell proliferation, alter gene expression and helps plants to recover from stress condition (Szabados and Savoure 2010).

Vigna radiata commonly known as mungbean is an important summer growing leguminous crop. It exhibit antioxidants, antimicrobial and anti-insecticidal activities (Samreen *et al*. 2017) because of high proteins, amino acids, oligosaccharides, and polyphenol content (Tang *et al*. 2014).

The present study was carried out to determine whether *Vigna radiata* has the ability to phytofilter toxic metal lead when grown hydroponically in sewage collected from sample site Sanwer road industrial area, Indore and also to investigate the effect of accumulated lead on *V. radiata* in terms of growth attributes and stress markers.

MATERIALS AND METHODS

Plant Material

Experiments on *Vigna radiata* (Mungbean) variety Samrat-139 was conducted in the Department of Biochemistry at Holkar Science College, Indore (M.P). Seeds of *Vigna radiata* were surface sterilized with 0.1% w/v aqueous solution of mercuric chloride for 5 min to prevent fungal growth (Kabir *et al*. 2008) and were kept for germination on moistened whatman filter paper placed in petriplates.

Experimental Design

Experimental set up was completely randomized and consisted of three different hydroponic systems including one control and each hydroponic system was replicated thrice. Three treatment solutions used are (a) Modified Hoagland nutrient media (Taiz and Zeiger 1998) served as control, (b) Sewage water sample collected from sample site Khan River, near Sanver road industrial area, Indore (M.P), (c) 50% diluted sewage water sample.

Phytofiltration System

Phytofiltration system consisted of *V. radiata* plants grown hydroponically in three treatment solutions; control, sewage, and 50% sewage. In experiment, plastic pots (its top covered by plastic net) saturated with specific treatment solution were taken for hydroponic culture. Pots are opaque to sunlight in order to prevent algae growth in the nutrient media. Seven-days old seedlings of *V. radiata* were transferred from petriplates to each plastic pot in such a way that roots of the seedlings were inserted through the holes (present in net) in to the nutrient solution and the shoot was above the net. Now all sets were cultured hydroponically using static solution culture method in such a way that it received ample sunlight and air. Plants harvested after intervals of 15-days were analyzed for their growth attributes and stress markers.

Sewage water analysis before and after hydroponic culture was performed. The concentration of toxic metal lead of *V. radiata* was determined by Atomic absorption spectroscopy.

Growth Attributes

Length of Root and Shoot

Root and shoot length of *V. radiata* plants were recorded by using the standard centimeter scale (Kabir *et al*. 2008).

Fresh weight and dry weight

For determining fresh weight, three plants were selected at random. Dry weight was determined after drying the plants in hot air oven at 80°C for 24 hours (Kabir *et al*. 2008). Fresh weight and dry weight of the plants were recorded using electrical balance.

Stress Markers

Malondialdehyde Estimation

The level of lipid peroxidation was measured in terms of MDA following the method of Hearth and Packer (1968). Concentration of MDA was calculated by using the extinction coefficient of 155 $nM^{-1}\,cm^{-1}$ at 532 nm using the formula: A= εcl. (A-Absorbance at specific wavelength, ε-Extinction coefficient, L-length of cell (1cm), C-concentration).

Peroxidase activity

The enzyme activity was assayed spectrophotometrically at 430 nm following the procedure of Summer *et al*. (1943). Specific activity of enzyme was expressed as units/min/mg protein considering one unit of enzyme as an increase in OD by 1.0 under standard conditions.

Proline Estimation

Proline precipitated as a proline-sulphosalicylic acid complex was made to react with ninhydrin under acidic conditions to obtain red color which was measured colorimetrically at 520nm (Bates *et al*. 1973).

μmoles of proline/g tissue =

$$\frac{\mu\text{g proline/ mL} \times \text{toluene (mL)}}{115.5\ \text{(M.W of proline)}} \times \frac{5}{\text{g sample}}$$

Atomic Absorption Spectroscopy (AAS)

Concentration of absorbed toxic metal of *Vigna radiata* was estimated by using Atomic Absorption Spectroscopy (AAS) in which plant samples were dried at 80°C overnight in hot air oven. Lead extract was prepared by dissolving 1gm dry ashes of *V. radiata* in 50 ml Aquaregia. For standard preparation, 2ml of standard lead was taken. Concentration of lead in extract was determined by using Analytical JENA NOVA 350 AA, which is based on the principle of Atomic absorption spectroscopy.

Statistical Analysis

Results were expressed as ± mean standard deviation (SD). Levels of significance were represented by $p > 0.05$ (not significant), $p < 0.05$ (significant), and $p < 0.01$ (highly significant).

RESULTS AND DISCUSSION

Lead Accumulation

In the present study, when *Vigna radiata* plants were raised hydroponically in sewage, plants absorbed and accumulated toxic metal lead (Pb) from sewage as revealed by the results of Atomic absorption spectroscopy (Table 1). These results indicate that *V. radiata* plant has the ability to phytofilter lead from sewage (Fig. 2), which is in line with Islam *et al*., (2012) who showed phytofiltration of arsenic and cadmium from waste water by using *Micranthemum Umbrosum*. Similarly, phytofiltration of arsenic from contaminated water by using *Hydrilla verticillata* (Srivastav *et al*. 2011), phytoremediation of Cd, Ni and Cr from industrial sludge by using Seindapsus *pictus Var argyaeus* (Latiff *et al*. (2013) were also studied. Patel *et al*. (2015) suggested that plants like Sunflower, Indian mustard, and Water Hyacinth have the ability to remove copper and lead from polluted water. Alia *et al*. (2015) revealed that Spinach (*Spinacia oleracea*) has the tendency to bio accumulate heavy metals from growth medium. Jaiswal *et al*. (2017) also showed that *Trigonella*

Table 1: Comparison of growth parameters, stress markers of *Vigna radiata* grown hydroponically using different treatment solutions

Sl. No.	Parameters	Control (Hoagland media)	Sewage water (A)	50% diluted sewage (B)	% change A	% change B
1	Root Length (cm)	11.00 + 1.0 (0.577)	7.33 + 0.58 a*(0.33)	3.67 + 0.58 a*, b*(1.154)	-33.33	-63.64
2	Shoot Length (cm)	19.67 + 0.58(0.33)	16.00 + 1a* (0.577)	9.00 + 3.46a*, b* (2.00)	-18.64	-54.24
3	Total length (cm)	30.00 + 7.55 (4.3)	21.33 + 2.08a$^{N.S}$(1.2)	14.67 + 1.53a*, b*(0.88)	-28.89	-51.11
4	Fresh Weight (g)	0.88 + 0.02(0.01)	0.83 + 0.04 a$^{N.S}$(0.02)	0.52 + 0.04a**,b**(0.021)	-5.30	-41.29
5	Dry Weight (g)	0.15 + 0.01(0.003)	0.14 + 0.01 a$^{N.S}$ (0.004)	0.08 + 0.01a**, b**(0.003)	-7.61	-45.65
6	Malondialdehyde (mM/g)	0.0015 + 0.0002 (0.0001)	0.002 + 0.001 a*(0.001)	0.0006 + 0.0004a**, b** (0.001)	33.33	-59.86
7	Peroxidase (units/ min/g)	15.1 + 2.00(1.3)	20.1 + 0.79 a* (0.55)	17 + 0.4a$^{N.S}$, b**(0.278)	33.11	12.58
8	Proline (µmoles/g)	5.37 + 0.17 (0.09)	5.88 + 0.17 a*(0.099)	7.79 + 62a**, b**(0.36)	9.68	45.16
9	Lead (ppm/g)	N.D	0.08	0.06	8	6

$^{N.S}$ indicates not significant (p>0.05), * indicates significant (p<0.05), ** indicates highly significant (p<0.01), N.D: Not detected. Parenthesis () indicates standard error; ***a****-indicates p value as compared to control,* ***b****- indicates p value as compared to sewage.*

Table 2: Sewage water analysis before and after growing *Vigna radiata* plants hydroponically

Sl. No.	Parameters	Sewage analysis before growing *V. radiata* plants	Sewage analysis after growing *V. radiata* plants
1	pH	7.70	7.67
2	Electrical conductivity (dSm^{-1})	4.66	2.14
3	Calcium (meL^{-1})	14.80	7.90
4	Magnesium (meL^{-1})	10.60	4.80
5	Sodium (meL^{-1})	19.00	8.10
6	Potassium (meL^{-1})	2.60	2.40
7	Carbonate (meL^{-1})	9.20	2.60
8	Bicarbonate (meL^{-1})	7.80	4.80
9	Chloride (meL^{-1})	18.40	8.40
10	Sulphate (meL^{-1})	12.10	5.20
11	Sodium Adsorption ratio (mmol^{L-1})	5.33	3.21

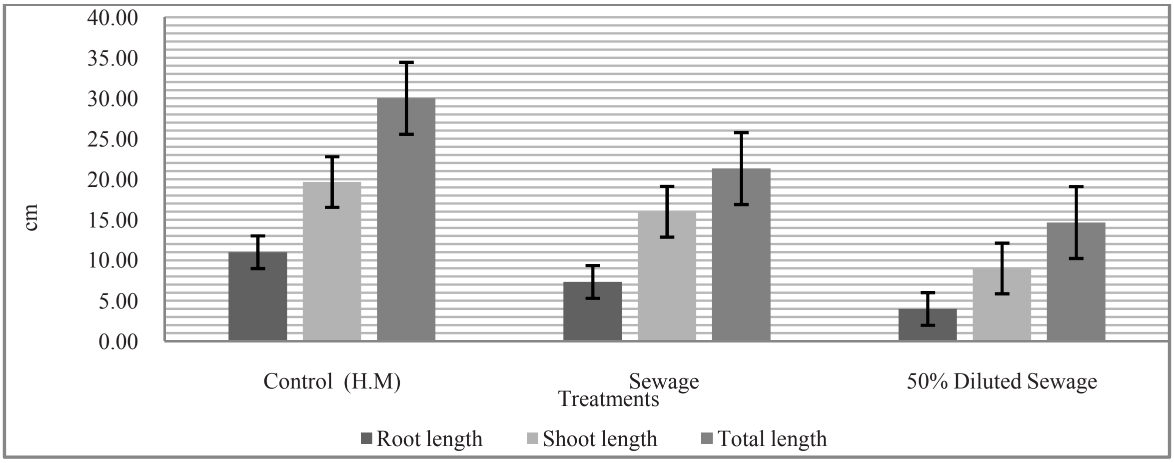
foenum-graecum has the potential to phytofiltrate cadmium from sewage.

Growth Attributes

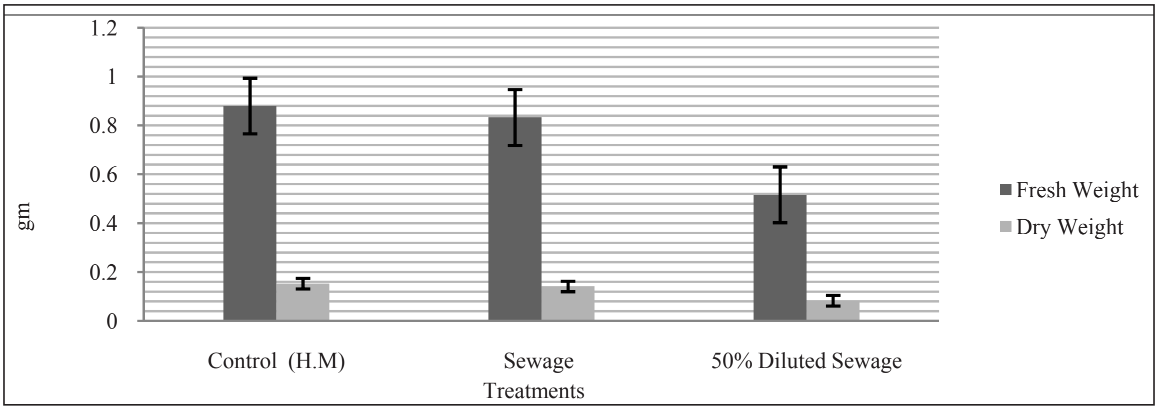
Root and Shoot length

Root and shoot length of *V. radiata* reduced significantly in both sewage and 50% diluted sewage. Total length reduced insignificantly in sewage and significantly in 50% diluted sewage (Graph 1)when compared to control. The root length reduced because of lead accumulation and might be due to the direct exposure of roots with toxic components of sewage. These results were in accordance with Verma and Dubey (2003) who showed that root length of 20-days-old rice seedlings significantly reduced up to 40% with increase in (0.5–1 mM) lead concentration.

According to Fahr *et al*. (2013), lead negatively affected growth and the branching pattern of roots by inhibiting cell division and elongation. Shoot length reduced because of the toxic effect of lead on metabolic activities (Sengar *et al*. 2008). It is possible that lead and free radicals disrupted tertiary structure of protein by binding with –SH group and inactivated enzymes involved in metabolic activities. This is in favor with Gupta *et al.* (2009) who revealed that free radicals generated due to heavy metal stress denature protein and negatively affect plant growth. Bharwana *et al*. (2016) suggested



Graph 1: Effect of different treatment on root-shoot and total length (cm) of hydroponically grown *Vigna radiata*



Graph 2: Effect of different treatment on Fresh-dry weight (gm) of hydroponically grown *Vigna radiata*

that lead enhance generation of free radicals in cotton (*Gossypium hirsutum L.*) seedlings and inhibit metabolic activities. John *et al*. (2009) also reported that growth of *Brassica juncea L.* reduced because of lead stress. It was reported by Lamhamdi *et al*. (2013) that lead chelates with mineral nutrients (Na, K, Ca, P, Mg, Fe, Cu and Zn) and reduces their bioavailability in wheat and spinach. Malar *et al*. (2014) revealed that lead chelates with nucleic acid and inhibits process of replication and transcription which ultimately cause reduction of plant growth.

Fresh and Dry weight

Fresh and dry weight reduced insignificantly in sewage and highly significantly in 50% diluted sewage when compared to control (Graph 2). Reduction in fresh and dry weight was due to the toxic impact of lead on rate of photosynthesis and solid (sugar and protein) content of *V. radiata*. Fresh weight might be reduced due to imbalance in water uptake of plant because of lead stress. Jayasri and Suthindhiran, (2017), suggested that fresh and dry weight of *Lemno minor* reduced significantly (80 %) due to toxic effect of Lead (6 mg/l) on two photosystems which reduced chlorophyll content of plant. Azad *et al*. (2011) reported that lead induce oxidative stress in Sunflower *(Helianthus annus L.)* and disturbs the biochemical process.

In the present study, growth attributes in terms of total length, fresh and dry weight of *V. radiata* insignificantly reduced when it was grown in sewage, while it significantly reduced in 50% diluted sewage (as shown in Fig. 1). Increased level of stress markers in *V. radiata* indicates oxidative stress however despite of oxidative stress the overall growth of *V. radiata* was affected insignificantly when compared to control. It might be due to plenty of available nutrients in sewage (Table 2), increased peroxidase and proline content which helped the plant to tolerate lead stress. In 50% diluted sewage, there was a significant decrease in growth when compared to sewage, which may be due to the lack of nutrients availability required for metabolic activities.

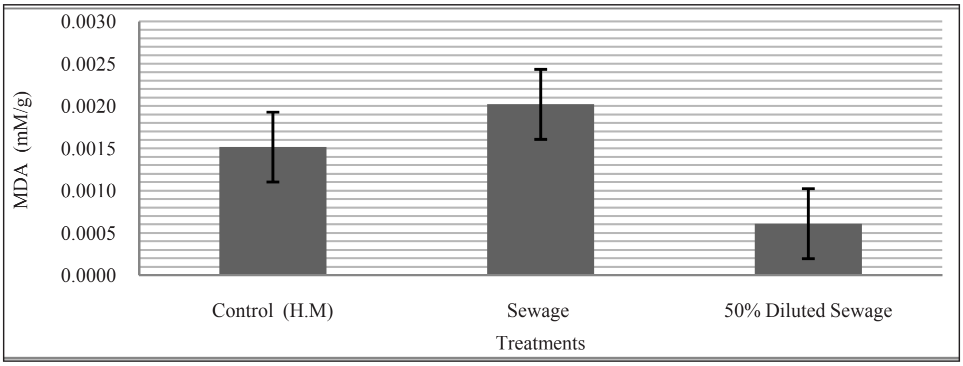
Fig. 1: Comparison between growths of *Vigna radiata* grown hydroponically in different treatment solutions

Malondialdehyde (MDA)

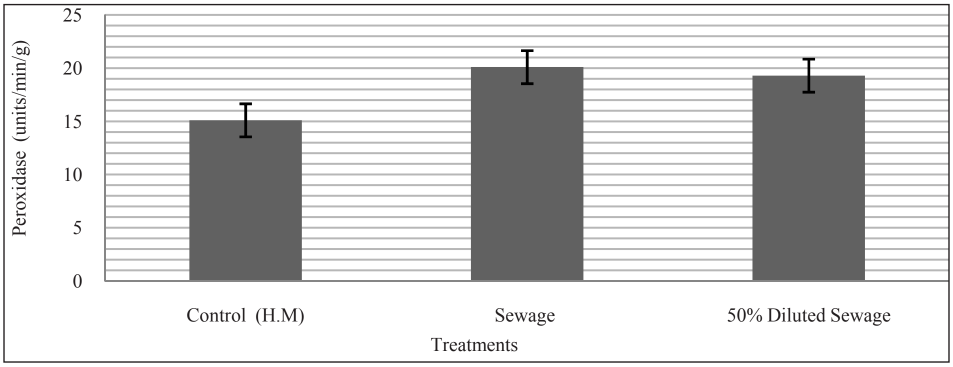
Malondialdehyde (MDA) level of *V. radiata* increased significantly in sewage, while it reduced significantly high in 50% diluted sewage when compared to control (Graph 3). In the present study, lead present in sewage was accumulated in hydroponically grown *V. radiata*. MDA level increased due to oxidative stress induced in the presence of lead. These results are in favor with Malar *et al*. (2014), who suggested that MDA level of water hyacinth increased due to lead stress. Similarly Singh and Agrawal, (2010) also showed that MDA level increased due to toxic components of sewage. According to Singh *et al*. (2017), lead is a toxic metal that impairs metabolic activities of plant and enhances generation of reactive oxygen species that induces oxidative stress. Reactive oxygen species like hydrogen peroxide (H_2O_2) cause peroxidation of PUFA and arachidonic acid in lipid membrane and enhances the level of MDA (Ayala *et al*. 2014). It was revealed by Gupta *et al*. (2009) that lipid peroxidation serves as one of the possible mechanism by which lead toxicity can be manifested in the plant tissue. In the present study, MDA level reduced in 50% diluted sewage when compared to undiluted sewage because of low loads of lead accumulation and increased proline content.

Peroxidase activity

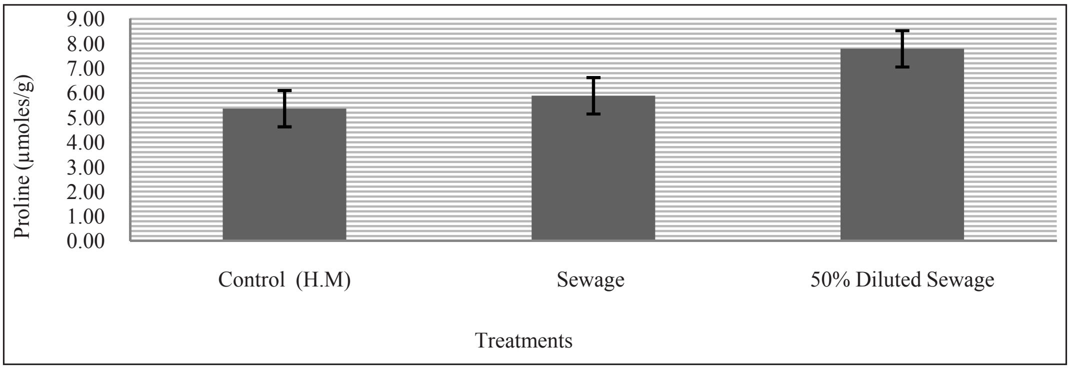
Peroxidase activity of *V. radiata* increased significantly in sewage and insignificantly in 50% diluted sewage when compared to those grown in control (Graph 4). Singh *et al.* (2010) suggested that toxic components present in waste water increased the peroxidase activity of plants when compared to ground water. Akee, (2016) revealed that peroxidase activity of



Graph 3: Effect of different treatments on MDA (mM/g) of hydroponically grown *Vigna radiata*



Graph 4: Effect of different treatment on Peroxidase (units/min/g) activity of hydroponically *Vigna radiata*



Graph 5: Effect of different treatment on Proline (µmoles/g) content of hydroponically grown *Vigna radiata*

Pharagmits australis increased to scavenges free radicals generated due to lead stress. Malar *et al*. (2014) also showed increased peroxidase activity (POX) in water hyacinth at higher lead (Pb) concentration and suggested that this plant has efficient antioxidative enzyme mechanism to tolerate lead (Pb) toxicity. Enzymatic antioxidants are important in plant defenses against heavy metals (Ciupa *et al*. 2016).

Fig. 2: Phytofiltration of lead by growing *Vigna radiata* (Mungbean) hydroponically in sewage

Fig. 3: Comparison between *Vigna radiata* grown in sewage and 50% diluted sewage

Free radicals generated due to lead stress, disturbs the metabolic activities through oxidative damage to cellular components (Dugar and Bafna, 2013, Emamverdian *et al*. 2015). Peroxidase serves as an intrinsic defense tool to resist oxidative damage against stress conditions (Aki *et al*. 2009). In the present study, oxidative stress was induced due to lead accumulation in *V. radiata* grown in sewage as indicated by increased malondialdehyde and peroxidase activity was enhanced to ameliorate lead toxicity. It is also possible that the mineral ions present in sewage may have stimulated peroxidase activity. Peroxidase activity insignificantly changed in 50% sewage when compared to control because of reduced oxidative stress due to dilution of toxic components of sewage.

Proline

Proline increased significantly in sewage and significantly high in 50% diluted sewage when compared to control (Graph 5). Similar increased proline content was also observed in *Triticum aestivum* due to cadmium stress (Nikolic *et al*. 2014), and in *Vicia faba* because of lead stress (Sharifa and Muriefah 2015). In plants grown under stress conditions, proline synthesizes from glutamic acid and contributes 80% of total amino acid pool. These mechanisms serve as an adaptive mechanism to reduce accumulated NADH and acidity (Britto *et al*. 2013; Ciupa *et al*. 2016). According to Szabados and Savoure, (2010), proline (an imino acid) binds with toxic metal ion due to its chelating ability and also scavenges free radicals to alleviate metal toxicity. Proline regulates normal range of free radicals, stabilizes biological membrane, reduces electrolyte leakage and thus prevents cell from oxidative burst (Lamhamdi *et al*. 2013). Proline also serves as a molecular chaperone that maintains proteins integrity, increases activities of different enzymes that help plants to tolerate stress conditions (Das and Roychudhary 2014). It may be possible that in sewage due to toxic impact of lead on carbohydrate biosynthesis, water uptake and in 50% diluted sewage due to inadequate availability of nutrients for carbohydrate biosynthesis, osmotic balance of *V. radiata* was disturbed and the need to maintain osmotic balance proline content increased. In sewage both peroxidase and proline increased but in 50% sewage only proline increased.

CONCLUSION

Vigna radiata absorbed lead from sewage and despite the accumulation of lead no significant change in growth attributes in terms of total length, fresh and dry weight was observed when compared to control. Increased Malondialdehyde (MDA) level indicates oxidative stress due to lead accumulation, while increased antioxidative stress markers proline and peroxidase (POD) activity shows that *V. radiata* has efficient scavenging mechanism to tolerate lead stress.

In *V. radiata* grown using 50% sewage, stress markers malondialdehyde and peroxidase reduced significantly along with the growth attributes like root-shoot length, fresh and dry weight when compared to undiluted sewage. These results indicate that diluted sewage was effective in reducing oxidative stress but it also adversely affected growth of the plants.

It was concluded that *Vigna radiata* has the potential to tolerate lead stress and can be used in phytofiltration of toxic metals from sewage. It was suggested that sewage contaminated with toxic components including lead should not be used for the irrigation of edible crops. Instead, it should be used for growing ornamental and flowering plants. 50% diluted sewage should be supplemented with growth promoting nutrients for its use in irrigation purpose.

ACKNOWLEDGEMENTS

We express our sincere thanks to Dr. Rooplekha Vyas, Principal and Prof. Dr. Angurbala Bafna, Head of Department of Biochemistry, Govt. Holkar Science College Indore (M.P.) for all the encouragement and for providing us necessary laboratory facilities.

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