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Enhancement of Biomass and Yield with Ni Uptake and Tolerance of *Brassica juncea* (cv. Pusa Bold) by Applying Inorganic Fertilizers in Soil Amended with Flyash and Wastewater

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Abstract The two waste products, flyash (FA) from thermal power plant and wastewater (WW) generated from urban population has assumed a big and important dimension in many countries including India. Harduaganj thermal power plant is source of flyash in Aligarh, famous for lock and nickel plating industries discharging huge amount of wastewater rich in heavy metals. Today both wastes have become an alternative source of chemical fertilizers for local farmers. In this paper, their utilization is discussed on the basis of results obtained in a pot experiment conducted in Department of Botany, while observing the growth, yield and nickel uptake of *Brassica juncea* (cv. Pusa Bold). The experiment was run with two levels of FA @ 10 and 20 t ha^{-1} and three doses of NPK @ 40:15:15, 60:30:30 and 80:45:45 kg ha^{-1}. Results showed that drymatter yield, seed yield and oil content of the crop tested was increased in both $FA_{10}WW$ and $FA_{20}WW$ as compared to crop grown under GW treatments and control having no FA, WW and NPK. The nickel uptake and translocation factor (Tf) showed the successive potential of Ni tolerance of *Brassica juncea* in soil amended with FA and irrigated with WW. The Tf was higher in FA+WW treatments than FA+GW and control. The overall results indicated that $FA_{20}N_{60}P_{30}K_{30}$ was found to be most suitable combination with assessment of yield and high oil content as well as Ni accumulation in plant parts. Therefore both wastes may compensate the fertilizers consumption and in addition be an alternative option of waste disposal.

Keywords Accumulation, Flyash, Wastewater, Indian mustard, NPK, Bioconcentration factor, Transfer factor, Tolerance index

1. Introduction

Agricultural lands practices for wastewater use is a one of the options for dealing with as a way of disposing it, where out of ~30 million tons of wastewater ~70% is consumed as an agricultural fertilizer and irrigation source[1], with data on previously, numerous studies founded that wastewater rich with valuable sources such as organic matter, macro and micro nutrients that required by the plant for fertility and productivity of soil and reduce fertilizer application[2] especially N and P required for ample growth to increase the crop yield[3-6], however high content of N, P and K strengthens its fertigation/ manural value of field crops[7].

Flyash (FA) also known as one of the major solid wastes produced as a heterogenous and complex byproduct resulting from coal combustion in thermoelectric power plants (TPPs). The utilization of fly ash instead of dumping it as a waste material can be both on economic and environmental grounds[8]. The application of FA on agricultural land realizes both benefits and negative effect in environment. Like wastewater, disposal of flyash is also a serious environmental problem. Unlike flyash application to agricultural land does not supply crop requirements of essential plant nutrients such as N and P. However the effect of flyash was more prominent and effective for agriculture when used with wastewater application.[9] analyzed the potential of SLASH (a mixture of sewage sludge, lime and flyash (63:1) on the biomass and yield of *Triticum* and *Sorghum* spp. which were enhances up to three fold. Therefore, application of two waste products on urban agriculture land throws a major challenge of disposal and management problem of ever increasing amount of both wastes in an environmentally sustainable manner inspite of meet the twin objectives of nutrition and irrigation inexpensively being a critical inputs in agriculture. But it is necessary to model the concentration-uptake-dose-response

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functions between the amended medium and crop plant. While all these aspects possible only after a proper planning of their safe use and care should be taken because an excess application might be a non-point source of contamination [10]. Presence of high concentration of heavy metals in WW and FA is one of the major limitations for its application on land which might pose heavy metal accumulation in the plant tissues, however some heavy metals such as Fe, Cu, Co, Ni, Mo and Zn are essential for plant growth and development upto a limit.

Among oil yielding *Brassica* species, *Brassica juncea* has been studied extensively for its tolerance to heavy metals and has been suggested as a good bioremediation of metal contaminated soil[11]. Plants have a range of potential mechanism at the cellular level that might be involved in the detoxification and thus tolerance to heavy metal stress by primarily avoid the build up of toxic concentration at sensitive sites within the cell and preventing the damaging effects.

Nickel (Ni) is a major environmental contaminants and one of the wide spread heavy metals, defined as ultra micronutrient. It is considered to be an essential for possibly all plant species[12][13] in small quantity (0.01 to 5 µg g^{-1} dry wt.), being important component of many enzymes especially urease[14], however, at elevated concentrations of this metal becomes toxic for majority of plant species.

The present study deals with the physico-chemical characterization of Aligarh city's wastewater and flyash and its impact on fresh and drymass yield, seed yield and oil parameters of important locally grown oilseed mustard crop with focused on quantify the level of Ni and their potential to translocation in soil and test crop grown in soil receiving both byproducts.

2. Materials and Methods

2.1. Pot experiment Layout and Treatments Pattern

Pot experiment was conducted with 7kg soil on *Brassica juncea* cv. Pusa Bold, during the winter season of 2011-2012 in the net house of Department of Botany, Aligarh Muslim University, Aligarh (27° 52' N latitude, 78°51' E longitude at an elevation of 187.45 m above the sea level).

The experiment was laid in completely randomized block design. Seeds of *Brassica juncea* cv. Pusa Bold were procured from IARI (Indian Agriculture Research Institute), New Delhi, India and disinfected with 0.01% aqueous solution of mercuric chloride followed by repeated washing with double distilled water and then dried in shade before sowing. Seeds were sown at the rate of 10/ pot. Every treatment was replicated thrice. After 7 days of sowing (DAS), thinning was done and two plants were left in each pot. Each replicate pot has a set of four pots. In each experiment there were seven treatments with two waters (wastewater and groundwater) and each replicated thrice. Therefore, comparative effect of WW and GW as well as two levels of FA @ 10 and 20 t ha^{-1} and three levels of NPK as urea, superphosphate and muriate of potash @ 40 kg N ha^{-1}, 15 kg P ha^{-1} and 15 kg K ha^{-1}, 60 kg N ha^{-1}, 30 kg P ha^{-1} and 30 kg K ha^{-1} and 80 kg N ha^{-1}, 45 kg P ha^{-1} and 45 kg K ha^{-1}, was observed to obtain the suitable combination of inorganic fertilizers, FA and water based on the growth performance *Brassica* cultivars and their heavy metal extracting capability with biomass production. The results were recorded by the study of shoot fresh and dry weight, leaf number and leaf area, seed yield, oil content and oil yield as well as Ni in leaf at 105 DAS namely fruiting stage and in seed at harvest. All experiments were carried out according to factorial randomized block design. To confirm the variability of data and validity of results, analysis of variance (ANOVA) was conducted. In order to determine whether differences among the treatments within respective DAS were significant as compared to control, Duncan's Multiple Range test was applied.

2.2. Sampling and Analysis of Water

Table 1. Physiochemical characteristics of ground water (GW) and wastewater (WW). All determinations in mg L^{-1} or as specified (except pH)

Characteristics	GW	WW
pH	6.9	7.9
EC (µ mhos cm^{-1})	711	840
Total solids (T.S.)	902	1209
Total dissolved solids (T.D.S.)	525	632
Total suspended solids (T.S.S.)	420	675
Dissolved oxygen	7.4	2.30
Biological oxygen demand (B.O.D.)	15.99	160.75
Chemical oxygen demand (C.O.D.)	35.20	119.19
Hardness	110.0	320.0
Magnesium (Mg)	17.48	128.17
Calcium (Ca)	23.91	41.48
Potassium	6.08	16.67
Sodium	16.36	46.67
Bicarbonate (HCO_3^-)	61.00	86
Carbonate (CO_3^-)	33.2	118.24
Chloride (Cl^{-1})	59.73	113.1
Phosphate (PO_4^-)	0.37	1.15
Nitrate nitrogen (NO_3–N)	0.74	1.23
Ammonium nitrogen (NH_3-N)	1.13	5.21
Nickel (Ni)	ND	0.375

Because Aligarh city is famous for lock and nickel plating factory so the heavy metals were also analyzed in collected wastewater. Wastewater was collected from the drain running along the Aligarh-Mathura road where it is already being used by local farmers, that is 05 km away from the department of Botany, A.M.U whereas tap water was the source of ground water. The 300 ml of wastewater was applied alternately and it is a mixture of sewage together with the wastewater from local industrial units. Wastewater was applied after 10 days of sowing to avoid adverse effect on seed germination. Samples of ground water and wastewater were collected in 2 liter plastic bottles and analyzed. The analysis of irrigation water was carried out according to[15]. The parameters studied were presented in table 1. The heavy metals analyzed using the mixture of $HClO_4$-HNO_3-H_2SO_4 for digestion, also presented in table 1.

2.3. Sampling and Analysis of Soil and Flyash

For each experiment soil samples were collected from the pots before sowing which was mixed thoroughly with farmyard manure and analyzed for following physio-chemical parameters. The soil samples were also collected after mixing of flyash and wastewater irrigation at harvesting of crop for the analysis of heavy metals. Flyash was collected from flyash pond of Harduaganj Thermal power plant, Kasimpur located at 14 km away from Aligarh city. In the laboratory, the soil/flyash sample was grinded with the help of mortar and pestle and passed through a 2 mm sieve, oven dried and used for the study of following physio-chemical characteristics of (Table 2). Total heavy metal concentration in soil/flyash before sowing and at harvesting (mg/kg) was determined by the *aqua-regia* acid digestion method[16].

2.4. Biomass Yield

The plants were ploughed out at 105 DAS (fruiting stage) and washed by deionized water followed by proper blotting between filter papers. Plants were dried in a hot air oven at 65℃ ± 2℃ for 72 h. The samples were weighed on an electronic top pan balance (KERN Compact Balance EMB, Germany) so as to obtain the biomass accumulation, which was expressed in g $plant^{-1}$. While yield was recorded at harvest period.

Table 2. Chemical characteristics of soil before sowing and of flyash before mixing in soil. All determinations in mg L^{-1} 1:5 (soil : water extract), except pH or as specified

Characteristics	soil	flyash
Texture	Sandy loam	-
CEC(meq $100g^{-1}$ soil)	2.92	-
pH	7.8	8.5
Organic carbon (%)	0.589	0.222
EC (μ mhos cm^{-1})	288	11.66
NO_3–N (g kg^{-1} soil)	0.292	0.060
Phosphorus (g kg^{-1} soil)	13.6	0.021
Potassium	21.00	15.00
Magnesium	31.42	19.56
Calcium	19.31	20.95
Sodium	12.02	16.13
Bicarbonate	19.33	13.42
Carbonate	78.29	70.14
Sulphate	17.66	26.25
Chloride	28.22	19.71
Nickel (Ni)	11.31	197.0

2.5. Heavy Metal Analysis

Dried plant sample then ground and passed through 1mm sieve and were further proceed for analysis of heavy metal concentration (mg/kg dry matter) through acid digestion. 1 g dried plant parts samples were heated with 10 ml of HNO_3, H_2SO_4 and $HClO_4$ mixture in 5:1:1 ratio at 80 ℃ until a clear was obtained[17]. The solution was filtered through Whatman no 42 filter paper and solution was diluted to 100 ml with DDW. The concentration of heavy metals (HMs) in filtrate was determined by using GBS; SenaAA model of double beam atomic absorption spectrophotometer (AAS).

Bio-concentration Factor (BCF) and Translocation Factor (TF): The BCF, TF, Ti and EF of HMs were calculated at the time of harvesting according to[18-21] respectively by the following formulas:

$$BCF = \frac{Metal\ concentration\ \ in\ leaf\ \ (mg/kg\ dw)}{Metal\ concentration\ \ in\ contaminated\ \ soils\ (mg/kg\ dw)}$$

$$TF = \frac{Concentration\ \ of\ metals\ \ in\ seed\ (mg/kg\ dw)}{Concentration\ \ of\ metals\ \ in\ coressponding\ \ \ leaf\ \ (mg/kg\ dw)}$$

$$Ti = \frac{Biomass\ \ or\ dry\ weig\,ht\ \ of\ treated\ \ plants\ \ (g/plant\ )}{Biom\,ass\ or\ dry\ weig\,ht\ of\ control\ \ plants\ \ (g/plant\ )}$$

$$EF = \frac{Concentration\ \ of\ metals\ \ in\ soil\ or\ plant\ \ parts\ \ at\ contaminated\ \ \ site)}{BConcentration\ \ of\ meta\,ls\ in\ soil\ or\ plant\ \ parts\ \ at\ uncontaminated\ \ \ site)}\ .$$

3. Results and Discussion

3.1. Biomass and Yield Production of Plant

Crop yields are generally limited by macro and micronutrients, since these are utilized by crops comparatively in larger quantity. Soil fertility can therefore be maintained by supplying these nutrients as fertilizers through wastewater and flyash. In the present study the GW and WW together with or without flyash along with 3 doses of NPK fertilization has the significant potential in agriculture as soil amendments was taken where WW irrigation and FA when added to soil, was found beneficial for *Brassica juncea* cv. Pusa bold up to 20 t ha^{-1} with low dose of NPK. Mean comparison showed that the use of wastewater in comparison with ground water results in increase of growth (Figure A, B, C, D) and yield (Figure E, F, G) of test crop. Similar results were also observed with positive response of various crops in wastewater application by[4] and[22]. The increase of shoot fresh weight, dry weight, leaf number, leaf area, yield and quality attributes of *B. juncea* cv. Pusa Bold expectedly may be due to availability of the amount of enough macro and micro nutritious elements present in wastewater viz., N, P, K, Ca, Mg, S, Cl etc, and flyash rich in oxides of Ca, B, Si, Al, Mg etc except N and little P[23], along with some heavy metals[24]. These major constituents required by plants for development and metabolic functions and might have played a cumulative role to enhanced growth and photosynthetic capacity which ultimately led to higher pod number, 1000 grain weight and finally the seed yield (Figure E).

The ameliorative effect of both wastes is also evident from the result obtained in the present study. Higher values in growth and yield parameters obtained when the nutrient through flyash was supplied at its optimum level. It is, therefore, logical to conclude that FA @ 20 t ha^{-1} with low dose of NPK (60, 30, 30 kg ha^{-1} respectively) enhanced the leaf production, leaf area, fresh and dry weight maximum when applied with wastewater, as higher dose fertilizers (80, 45 and 45 kg ha^{-1} NPK) could not enhance them. Present findings also strengthen the earlier findings[25]. The leaf area is the most important because larger green surface area required for photosynthetic activity which ultimately enhanced the seed yield. The leaf area is also dependent upon the supply of sulphur element because it also pointed out that the application of nitrogenous fertilizers such as urea is ineffective in absence of sulphur as its deficiency reduces the leaf area, oil content and oil yield. While such effects were not observed in present study due to the application of wastewater which contains the sulphur in the form of suphates ions. Among the oilseed crops, oil related parameter is on place of importance, and depends upon sulphur nutrition requirement[26], as it is a key ingredient of oil[27]. The interactive effect of NPK+FA $\times$ GW and WW irrigation was significant over control in almost of all the parameters studied except seed yield of *B. juncea* cv. Pusa Bold. The mean values showed that WW $\times$ $N_{60}P_{30}K_{30}FA_{20}$ was superior over all other combinations including control registered an increase of 106.06%, 93.48%, 16.94% fresh weight, leaf area and oil content respectively. Optimum seed yield was obtained again by the treatment $N_{60}P_{30}K_{30}FA_{20}$ while a yield advantage is not obtained due to combined application of NPK+FA with GW and WW was recorded to be non- significant at $p< 0.05$. It was also noted from data, we can say that with $GWN_{80}P_{45}K_{45}FA_{20}$ was equaled by that of half dose of $N_{40}P_{15}K_{15}FA_{20}$ with WW, similarly the half dose of this NPK with GW (i,e $GWN_{40}P_{15}K_{15}FA_{10}$) was equaled by of the irrigation of WW alone, indicating the wasteful consumption of higher dose of NPK may be removed by the utility of WW for providing the essential nutrients and lowering the fertilizers requirement.

Nickel (Ni) accumulation:

The total content of Ni was considerably higher in WW, FA @10 and 20 t ha^{-1} with all levels of NPK than control values. As the treatments levels increased, Ni concentration was also increased in leaf and seed of plant. On contrary to all above parameters, $WWN_{80}P_{45}K_{45}FA_{20}$ found to responsive dose to increase the metals content in both part of plant. The Ni content was ranged from 4.56-19.23 mg kg^{-1} DW in leaf while in seed it ranged from 0.286-1.669 mg kg^{-1} DW (Figure 2). It was also proved by[28] and recently,[29] have reported that the accumulation of metals in the plant of *Vigna radiata* increased with increasing FA amendment. It may be due to large amount of metal ions present in wastewater and flyash. Fortunately their concentration were within the permissible limit[30], making their use of nutritionally sustainable even FA at high dose i.e 20 t ha^{-1}.

The range of Ni was within toxic limit which were consonance with earlier finding of[31] reported that the plant of *B. juncea* was suitable for the phytoextraction of Ni from flyash contaminated or amended soil.

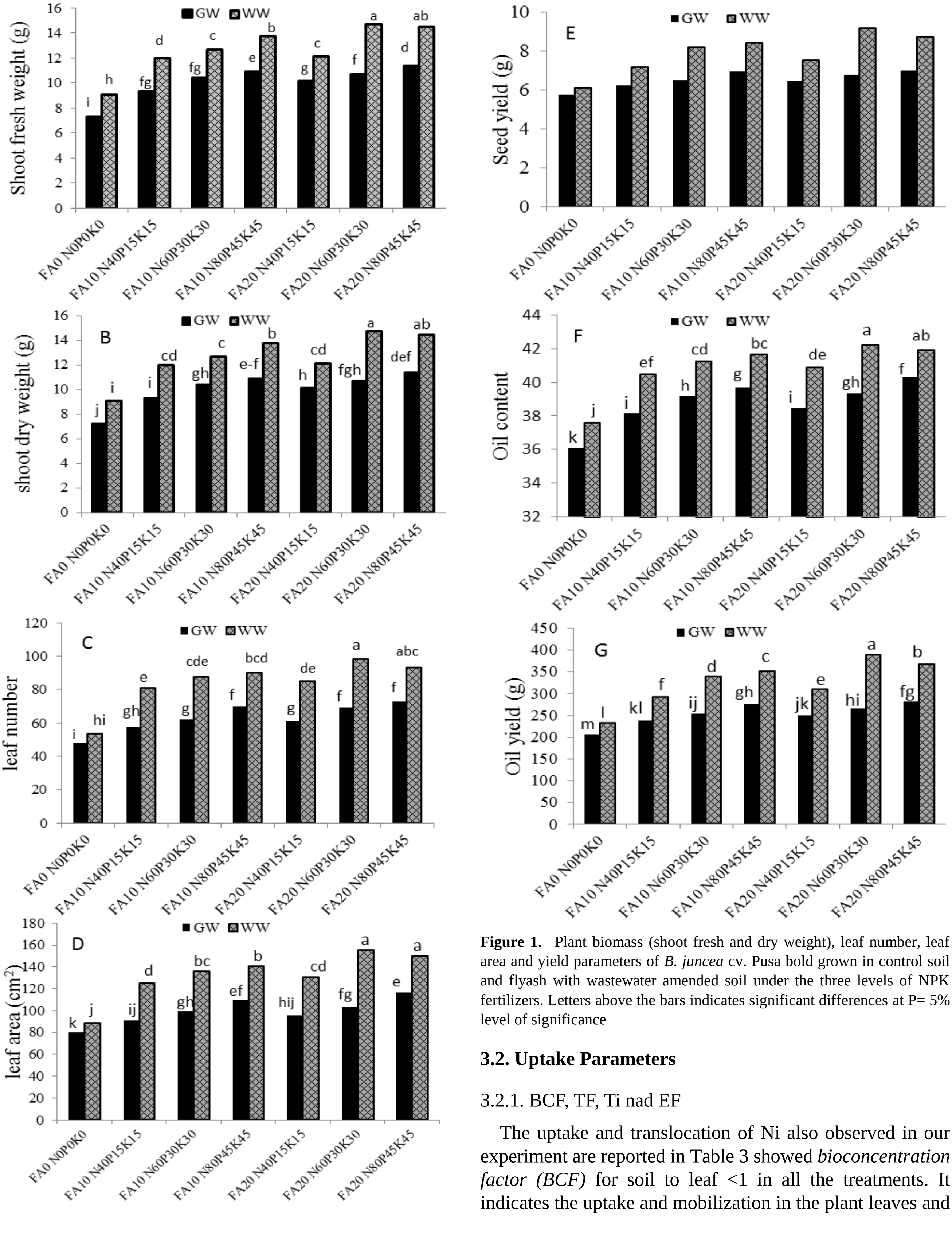

Figure 1. Plant biomass (shoot fresh and dry weight), leaf number, leaf area and yield parameters of *B. juncea* cv. Pusa bold grown in control soil and flyash with wastewater amended soil under the three levels of NPK fertilizers. Letters above the bars indicates significant differences at P= 5% level of significance

3.2. Uptake Parameters

3.2.1. BCF, TF, Ti nad EF

The uptake and translocation of Ni also observed in our experiment are reported in Table 3 showed *bioconcentration factor (BCF)* for soil to leaf <1 in all the treatments. It indicates the uptake and mobilization in the plant leaves and their storage in the aerial part biomass were comparatively less required for phytoremediation and also indicates that plants had difficulties in mobilizing Ni. It may be due to simultaneous use of FA and WW is beneficial from the point of view that and FA reduce heavy metals availability[32][10] by provides oxide of Ca(CaO) which is very important constituent forms $Ca(OH)_2$ helps in stabilizing or neutralizing the acidic environment[33] of wastewater irrigated soil by maintaining pH of WW because metal uptake and translocation basically linked to pH and organic matters[34].

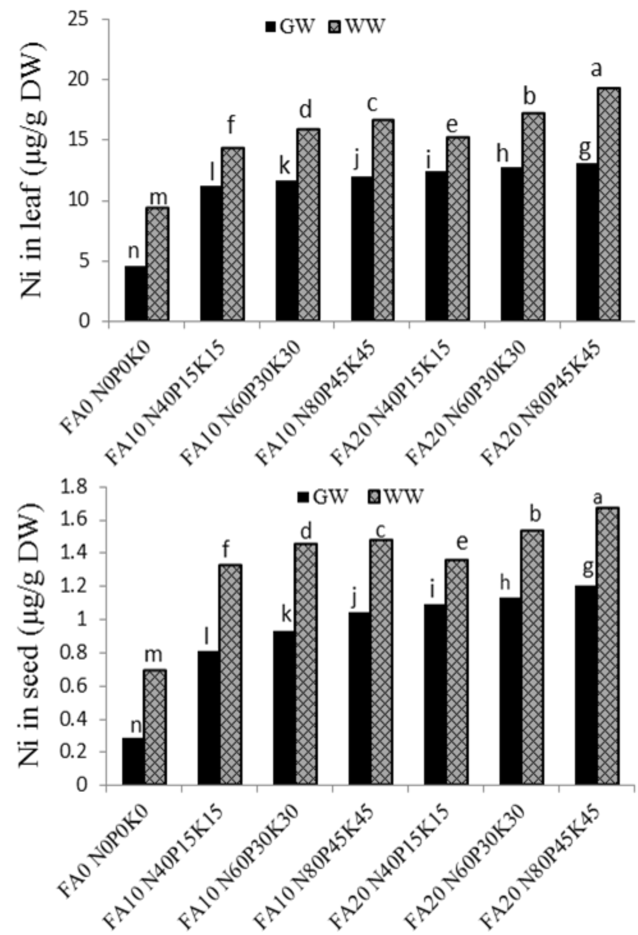

Figure 2. Nickel (Ni) content of *B. juncea* cv. Pusa bold grown in control soil and flyash with wastewater amended soil under the three levels of NPK fertilizers. Letters above the bars indicates significant differences at P= 5% level of significance

However another factor *translocation factor (Tf)* showed the values > 1 in almost treatments indicated the translocation of Ni from leaves to seeds was significant that proved the efficiency of *B. juncea* cv. P. Bold to translocation rate was better. The *tolerence index (Ti)* most important parameter showed values >1 reflects net increase in biomass with Ni uptake significantly indicated *B. juncea* cv. P. bold have developed tolerance/ability to survive in a soil that is toxic to other plant, therefore, proved favorable for phytoremediation[35, 36]**.** The *enrichment factor (Ef)* in contaminated soil, leaf and seed parts of *B. juncea* cv. Pusa Bold recorded the values was greater than one (1) which indicated higher availability of Ni in soil supplied with flyash and wastewater. It reflects increasing in accumulation and transfer of Ni in leaf and seeds. This was also agreed with the finding of[21][37], showed increasing the accumulation in plant species grown on contaminated soil. In case of leaf to leaf, seed to seed EF was maximum in the $FA_{20}N_{80}P_{45}K_{45}$WW recorded 4.22 and 5.84 respectively. While EF in both the edible parts of *B. juncea* cv. Pusa Bold may be proved an important criterian for the selection of this crop as it can be grown/cultivated by farmers in a field having higher levels of metals in soil contaminated or receiving industrial wastewater. However, the results of present study proved the ability to higher Ni accumulation from soil supplied with flyash and wastewater. The plants are adopted to detoxify Ni by varied mechanisms. The plasma membrane acts as a first defense line that decreases the influx or increase the exclusion of metal from the cell. Moreover, Ni inside the cell is chelated by the phytochelatins, metallothionine and nicotianamine which is sequested or compartmentalized into vacuole, where it is less toxic[38]. The sequestration in the vacuolar compartment is an important process, which excludes them from cellular sites where processes such as cell division and respiration occur, thus proving to be as effective protective mechanism[39-41]. According to[42][43], Ni is mobile in plants and accumulates readily in seeds, as well. Ni could be transported as a nickel-citrate complex[41] or as a nickel-peptide complex or as a nickel-histidine complex[44] to ensure high mobility of Ni within the plant. The Ni tolerant proteins TgMTPIs from the Ni hyperaccumulator species *T. goesingense* have been suggested to be responsible for metal ion accumulation in the shoot vacuoles of this plant[45].

Table 3. Transfer Factors/ Mobilization and Tolerance Index of Brassica juncea cv. Pusa bold Under Different Treatments with Flyash and Wastewater Contaminated and Uncontaminated Soil

Treatments	Ni Level (mg/kg)	BCF Soil to leaves	Tf Leaves to seed	Ef Leaf to leaf	Ef Seed to seed	Ef Soil to soil	Ti
Uncontaminated soil	11.31	-	-	-	-	3.77	-
Contaminated soil	42.69	-	-	-	-	-	-
$FA_0N_0P_0K_0$ GW	-	-	0.286	-	-	-	-
$FA_{10}N_{40}P_{15}K_{15}$ GW	-	0.263	0.812	2.465	2.84	-	1.277
$FA_{10}N_{60}P_{30}K_{30}$ GW	-	0.272	0.933	2.550	3.26	-	1.431
$FA_{10}N_{80}P_{45}K_{45}$ GW	-	0.280	1.044	2.621	3.65	-	1.494
$FA_{20}N_{40}P_{15}K_{15}$ GW	-	0.291	1.095	2.728	3.83	-	1.392
$FA_{20}N_{60}P_{30}K_{30}$ GW	-	0.298	1.135	2.794	3.97	-	1.468
$FA_{20}N_{80}P_{45}K_{45}$ GW	-	0.305	1.208	2.860	4.22	-	1.564
$FA_0N_0P_0K_0$ WW	-	0.217	0.688	2.033	2.41	-	1.244
$FA_{10}N_{40}P_{15}K_{15}$ WW	-	0.334	1.326	3.127	4.64	-	1.638
$FA_{10}N_{60}P_{30}K_{30}$ WW	-	0.372	1.455	3.485	5.09	-	1.738
$FA_{10}N_{80}P_{45}K_{45}$ WW	-	0.390	1.478	3.647	5.17	-	1.883
$FA_{20}N_{40}P_{15}K_{15}$ WW	-	0.355	1.354	3.322	4.73	-	1.661
$FA_{20}N_{60}P_{30}K_{30}$ WW	-	0.403	1.533	3.774	5.36	-	**2.016**
$FA_{20}N_{80}P_{45}K_{45}$ WW	-	**0.450**	**1.669**	**4.217**	**5.84**	-	1.982

4. Conclusions

It was concluded that from the experimental results application of WW seems to pose no harm to *Brassica juncea* cv. Pusa Bold and instead could supplement, if not fully at least partly, the nutrient requirement of the crop leading to the reduction in fertilizers use and also saving the fresh water. FA @ 20 t ha^{-1} proved optimum/balanced dose with low dose of NPK ($N_{60}P_{30}K_{30}$) instead of use with higher dose ($N_{80}P_{45}K_{45}$). All the attributes increased in both levels 10 t ha^{-1} and 20 t ha^{-1} of FA with GW and WW and better over control therefore both levels of FA could be applied.

This study also gave a conclusion on research as well as economy point of view it can be recommended that farmers can grow *B. juncea* cv. Pusa Bold for more seed yield, oil yield as well as for biomass yield with successive heavy metal remediation efficiency in the field/land contaminated with these two by product wastes.

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