
Phytoremediation of heavy metals by *Trifolium alexandrinum*Hazrat Ali¹, Muhammad Naseer², Muhammad Anwar Sajad²

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doi:10.6088/ijes.00202030031

ABSTRACT

The release and persistence of toxic heavy metals into the natural environment is a serious concern especially in urban areas. The problem of heavy metal pollution is gaining momentum from year to year as more and more amounts of heavy metals are extracted from their ores and released into the environmental segments (water, air and soil) during processing or afterwards. Heavy metals are essentially nonbiodegradable and therefore accumulate in the environment and subsequently find their way into the food chains. Contamination of food chains by toxic heavy metals is an unwanted outcome of industrialization and unsustainable development. This contamination is a risk to the health of all organisms including humans. Entrance of toxic heavy metals (through absorption, inhalation and ingestion) into the human body beyond threshold limits causes many diseases and health abnormalities. Therefore, effective remediation of heavy metal pollution is a top priority. The different physico-chemical methods used for this purpose generally suffer from serious limitations. Phytoremediation is seen as an alternative green solution to the problem. The present study reports phytoremediation of Cd, Pb, Cu, and Zn by *Trifolium alexandrinum*, which is a suitable candidate plant species for this purpose. *T. alexandrinum* was grown in a simulated heavy metal-contaminated soil. Root bioconcentration factor values of *T. alexandrinum* for Zn, Pb, Cu and Cd were 4.242, 1.544, 1.071, and 0.604 respectively.

Keywords: Phytoextraction; heavy metals; soil pollution; *Trifolium alexandrinum*; phytoremediation

1. Introduction

The mobilization of heavy metals by man (through mining from ores and processing for different applications) has led to the contamination of different environmental segments by these elements. By contaminating food chain, these elements pose a risk to environmental and human health. As a result of their release and presence in the ecosystems, these pollutants are accumulated by living organisms in their bodies and subsequently biomagnified as they pass from one trophic level to the next. Since man also is at the top of food chain, he is vulnerable to heavy metal pollution.

Regarding the role of heavy metals in living systems, they are divided into two classes: essential and non-essential. Essential heavy metals are those, which are needed by living organisms for their growth, development and physiological functions like Mn, Fe, Ni, Cu and Zn (Gohre and Paszkowski, 2006) while non-essential heavy metals are those, which are not needed by living organisms for any physiological functions like Cd, Pb, Hg and As (Mertz,

1981; Suzuki and Sano, 2001; Bidar et al., 2006; Peng et al., 2009). Higher levels of heavy metals disturb the normal physiology and biochemistry of living systems. The most dangerous heavy metals are Pb, Hg, As, Cd, Sn, Cr, Zn and Cu (Wright, 2007; Gosh, 2010). Among these, Cd and Pb are the most dangerous metals for human health (Sekara et al., 2005). Cd is considered as the most serious pollutant of the modern age (Singh et al., 2009). Cd concentrations above the threshold limit values have been found to be carcinogenic, mutagenic and teratogenic for a large number of animal species (Degraeve, 1981). Cd has also been implicated as an endocrine disruptor (Awofolu, 2005). Pb has been found to be responsible for quite a number of ailments in humans such as chronic neurological disorders especially in fetus and children. This eventually results in behavioral and attitude changes with progressive retardation (Awofolu, 2005). Pb-poisoning in children causes neurological damage leading to reduced intelligence, loss of short-term memory, learning disabilities and coordination problems (Padmavathiamma and Li, 2007).

The concentrations of heavy metals increase in the environment from year to year (Govindasamy, 2011). Therefore decontamination of heavy metal-contaminated soils is very important for maintenance of environmental health and ecological restoration. Different physical and chemical methods are used for this purpose with each method having its merits and demerits. However, physical and chemical methods are generally considered as destructive, expensive, labor-intensive and causing secondary problems (Padmavathiamma and Li, 2007; Wu et al., 2010). In comparison, phytoremediation is a novel, less expensive, efficient, environment- and eco-friendly remediation strategy with good public acceptance (Turan and Esringu, 2007; Singh et al., 2009; Saier and Trevors, 2010; Revathi et al., 2011). Phytoremediation is the use of green plants for decontamination of polluted sites (soils and waters). It can be used for heavy metals and radionuclides as well as for organic pollutants. Phytoremediation technology is a recent technology. Most research efforts are focused in this field since the last two decades (1990 onwards). Different techniques of phytoremediation include phytoextraction, phytofiltration, phytostabilization, phytovolatilization and phytodegradation (Alkorta et al., 2004). Phytoextraction is the uptake of elements (heavy metals) or compounds (xenobiotics) from growth media by plant roots and their translocation to the aerial parts (stem and leaves). It is the best approach to remove contaminants primarily from soil and isolate them without destroying the soil structure and fertility (Gosh and Singh, 2005). An additional advantage of phytoextraction is that during this practice, plants cover the soil and thus, erosion and leaching are minimized (Jadia and Fulekar, 2009). For phytoremediation, two approaches are used: (1) The application of hyperaccumulators (such as *Thlaspi caerulescens* or *Alyssum bertolonii*) producing a relatively low amount of above-ground biomass but accumulating high amounts of one or more elements (2) The application of high biomass producing plants characterized by lower ability to accumulate target elements where total uptake of elements is comparable to that of hyperaccumulators due to high yield of above-ground biomass (Tlustos et al., 2006). Hyperaccumulators are defined as “plant species whose shoots contain $> 100 \text{ mg Cd kg}^{-1}$, $> 1000 \text{ mg Ni, Pb and Cu kg}^{-1}$ or $> 10000 \text{ mg Zn and Mn kg}^{-1}$ (dry weight) when grown on metal rich soils” (Baker and Brooks, 1989). As of 2010, more than 400 plant species have been identified as metal hyperaccumulators (Wu et al., 2010; Mudgal et al., 2010). Grasses have been more preferable in use for phytoaccumulation (phytoextraction) than shrubs or trees because of high growth rate, more adaptability to stress environment and high biomass (Malik et al., 2010). Screening hyperaccumulators and accumulators is a key step in the phytoremediation of soils contaminated by heavy metals (Zhang et al., 2010). The use of hyperaccumulators to decontaminate polluted soils might result in production of a bio-ore of some commercial value to recoup some of the costs of soil remediation (Brooks et al., 1998). Phytoremediation

technology is interdisciplinary in nature and requires input of knowledge from chemistry, botany and soil microbiology. The present study was designed to investigate the phytoextraction of four selected heavy metals (Cd, Pb, Cu and Zn) from simulated polluted soil by *Trifolium alexandrinum*. *T. alexandrinum* is an herb belonging to the family Fabaceae. It is cultivated as a fodder crop for cattle. It was selected because it is fast-growing, resistant to pollution loads, produces high biomass and above all offers multiple harvests in a single growth period.

2. Materials and Methods

2.1 Experimental Plant

In this study, *T. alexandrinum* was used for phytoextraction of Cd, Pb, Cu and Zn. The seeds of the plant were obtained from the local market.

2.2 Phytoextraction Experiments

Phytoextraction experiments were carried out in pots in greenhouse. For this purpose, the soil was passed through a 1 mm sieve and 6.5 kg soil was put into each pot. Pots were divided into five groups i.e., Cd-contaminated, Pb-contaminated, Cu-contaminated, Zn-contaminated, and control (to which no metal was added). The metals were added to the soil as their water soluble salts in the form of their aqueous solutions. Thus Cd was added as $\text{CdCl}_2 \cdot 2 \frac{1}{2} \text{H}_2\text{O}$, Pb as $\text{Pb}(\text{NO}_3)_2$, Cu as $\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$, and Zn as $\text{ZnSO}_4 \cdot 7 \text{H}_2\text{O}$. The concentration of metals added was 100 mg metal per kg of soil (100 ppm). Ten seeds of *T. alexandrinum* were put into each pot. For each metal three pots were used (triplicate experiment). After germination, the seedlings were allowed to grow in the same pots until maturity. After 98 days, the mature plants were uprooted and separated into roots, stem and leaves. These parts i.e., roots, stem, and leaves were used for the analysis of accumulated heavy metals.

2.3 Analysis of Heavy Metals in Plants

For this purpose, each plant part was thoroughly washed with tap water and then with distilled water in order to remove dust and soil particles. The clean plant parts were dried in an oven at 80°C for 48 hours. Then the samples were digested according to Awofolu (2005): 0.5 g sample of the plant part was taken into a 100 mL beaker. 5 mL concentrated (65%) HNO_3 and 2 mL HClO_4 were added to it and heated on hot plate until the digest became clear. The digest was allowed to cool and then filtered through a Whatman filter paper. The filtrate was collected in a 50 mL volumetric (measuring) flask and diluted to the mark with distilled water. The filtrate was used for the analysis of heavy metals (Cd, Pb, Cu, and Zn) by Atomic Absorption Spectrophotometer (AAS-700, Perkin-Elmer, USA) using acetylene/air as gas mixture. The lamp wavelength (λ) for Cd, Pb, Cu, and Zn was 228.8 nm, 283.3 nm, 324.8 nm, and 213.9 nm respectively. As mentioned previously, each experiment was run in triplicate. Results are shown as mean $\pm$ Standard Error.

2.4 Calculation of Bioconcentration and Translocation Factors

Bioconcentration factor (BCF) indicates the efficiency of a plant in up-taking heavy metals from soil and accumulating them into its tissues. It is a ratio of the heavy metal concentration in the plant tissue (root, stem or leaves) to that in soil. It is calculated as follows (Zhuang et al., 2007).

$$BCF = \frac{C_{\text{harvested tissue}}}{C_{\text{soil}}}$$

where $C_{\text{harvested tissue}}$ is concentration of the target metal in the plant harvested tissue (roots, stem or leaves) and C_{soil} is concentration of the same metal in soil. Bioconcentration factor can also be calculated in percent according to the following equation (Wilson and Pyatt, 2007).

$$BCF (\%) = \frac{C_{\text{plant tissue}}}{C_{\text{soil}}} \times 100 \%$$

Translocation factor (TF) indicates the efficiency of the plant in translocating the accumulated heavy metals from roots to shoots. It is a ratio of the concentration of the heavy metal in shoots (stem or leaves) to that in its roots. It is calculated as follows (Padmavathiamma and Li, 2007; Adesodun et al., 2010).

$$TF = \frac{C_{\text{shoots}}}{C_{\text{roots}}}$$

2.5 Statistical Analysis

Each experiment was conducted in triplicate ($n = 3$). Results are shown as mean $\pm$ standard error. Experimental data were analyzed using statistical software SPSS 16.0. Heavy metal concentrations in control and experimental were compared using Paired-Samples T Test.

3. Results and Discussion

3.1 Phytoextraction of Cd

The accumulation of Cd in different tissues of *T. alexandrinum* is shown in Figure 1.

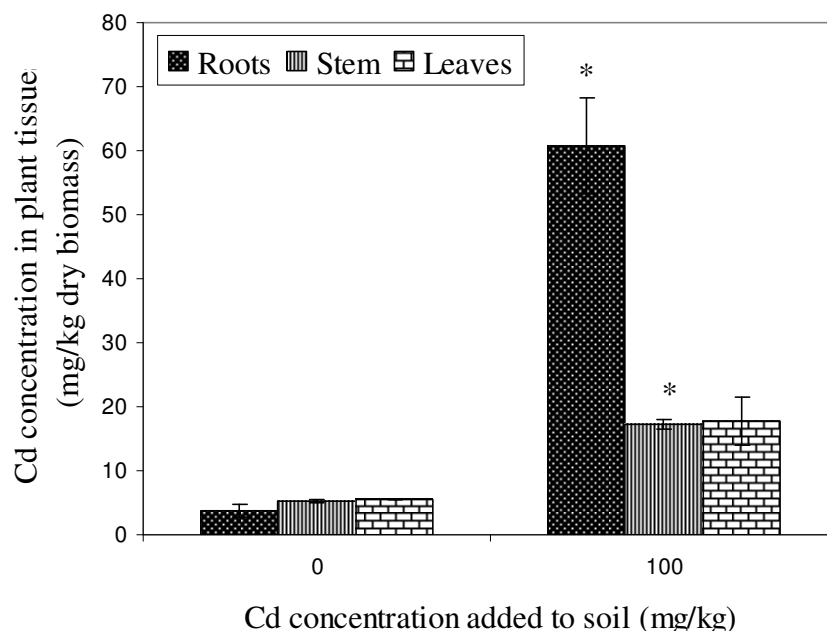


Figure 1: Phytoextraction of Cd by *T. alexandrinum*

Error bars show standard error ($n = 3$)

*Mean values are significantly different from their respective controls at $p < 0.05$ according to Paired-Samples T test.

The concentrations of Cd in roots and stem of *T. alexandrinum* were significantly higher ($p < 0.05$) in Cd-treated plants than in control plants. However, in case of leaves, the difference was not significant at $p < 0.05$. The concentrations of Cd in the three parts of Cd-treated plants were in the order: roots > leaves > stem. This sequence is commonly observed for accumulation of heavy metals in plants. According to Smical et al. (2008), different plant parts contain different quantities of heavy metals with the highest ones being contained in roots and leaves. This is because heavy metals are absorbed by roots from soil solution and later on translocated to leaves (through xylem vessels) where they are deposited in vacuoles. Stem is only a traffic way for this journey. According to Rahmanian et al. (2011), the maximum shoot Cd concentrations for couch grass, alfalfa and millet were 23.6, 13.4 and 15.1 mg kg⁻¹ dry mass respectively at a total soil Cd concentration of 108 mg kg⁻¹, which are comparable to our results.

3.2 Phytoextraction of Pb

The accumulation of Pb in the different tissues of *T. alexandrinum* is shown in Figure 2.

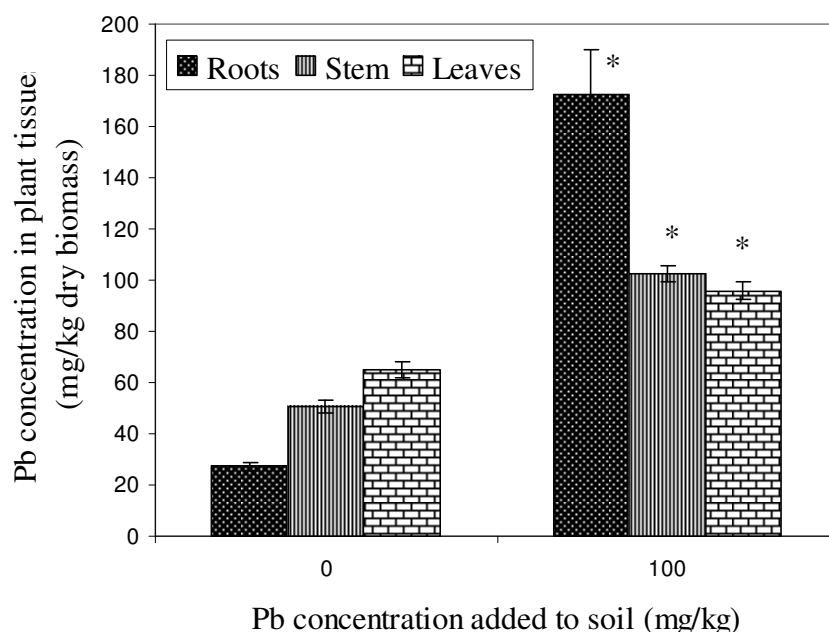


Figure 2: Phytoextraction of Pb by *T. alexandrinum*

Error bars show standard error (n = 3)

*Mean values are significantly different from their respective controls at $p < 0.05$ according to Paired-Samples T test.

Pb concentrations in all tissues of *T. alexandrinum* were significantly higher ($p < 0.05$) for Pb-treated plants compared to the control plants. Pb concentration was more in roots compared to both stem and leaves in case of Pb-treated plants. This is in agreement with the findings of Soleimani et al. (2009) who state that the amounts of accumulated Pb in tall fescue and Bermuda grass (*Cynodon dactylon*) were higher in roots compared to the shoots.

3.3 Phytoextraction of Cu

The accumulation of Cu in the different tissues of *T. alexandrinum* is shown in Figure 3.

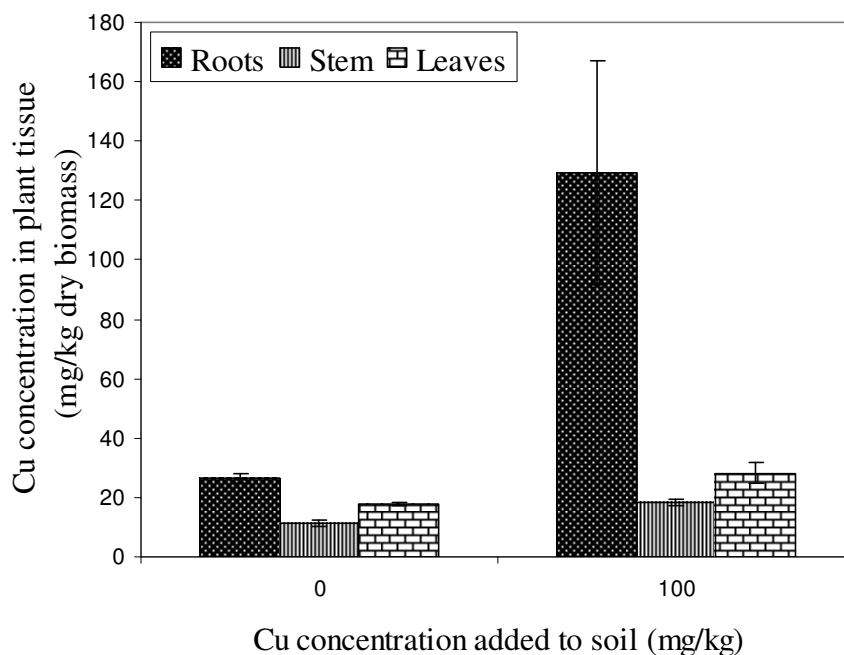


Figure 3: Phytoextraction of Cu by *T. alexandrinum*
Error bars show standard error (n = 3)

Unlike for Cd and Pb, the concentrations of Cu in the tissues of *T. alexandrinum* were significantly not different (at $p < 0.05$) for Cu-treated and control plants. Cu concentrations in the tissues of Cu-treated plants were in the order: roots > leaves > stem. This is supported by the results of Hajiboland (2005) who reported that metal amounts in roots were higher than those in the shoots.

3.4 Phytoextraction of Zn

The accumulation of Zn by the different tissues of *T. alexandrinum* is shown in Figure 4. Like for Cu, the concentrations of Zn in the tissues of *T. alexandrinum* are significantly not different (at $p < 0.05$) for Zn-treated and control plants. The concentrations of Zn in the different tissues of Zn-treated plants were in the order: roots > leaves > stem. Of the four studied metals (Cd, Pb, Cu, and Zn), *T. alexandrinum* plants accumulated more Zn in their roots. This trend has been reported by other authors as well using other plants. For example, Peralta et al. (2001) stated that alfalfa plants accumulated significantly more Zn in the roots than other metals. Gbaruko and Friday (2007) also reported that Zn had the highest concentration in the flesh of all the fauna and flora samples analyzed.

3.5 Bioconcentration Factors

Bioconcentration factors of *T. alexandrinum* for the selected heavy metals are shown in Table 1. Results are based on the total soil concentrations of the concerned metals (background concentrations + concentrations added to soil). Values of background concentrations of the selected heavy metals in the used soil were taken from a previous recent study (Sajad, 2010).

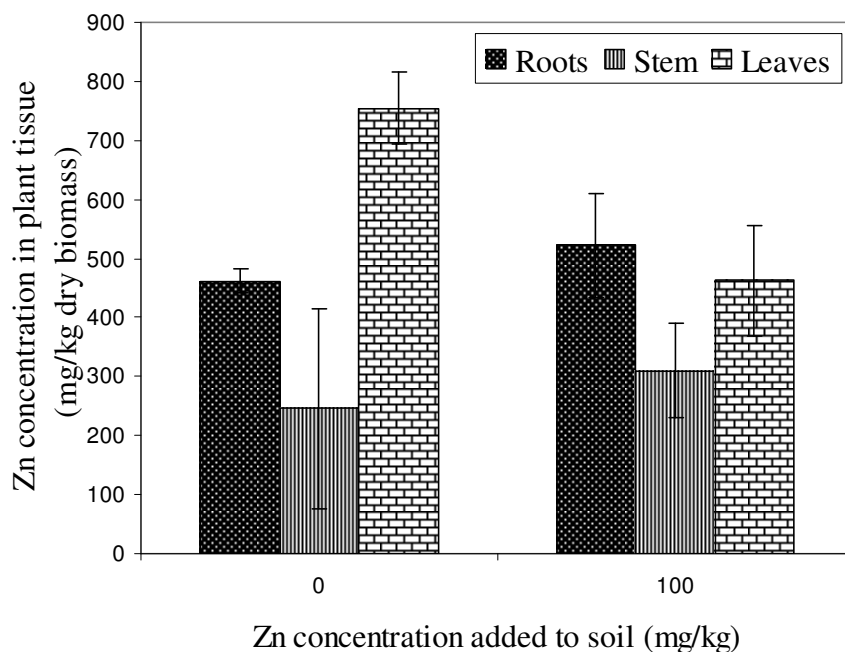


Figure 4: Phytoextraction of Zn by *T. alexandrinum*
Error bars show standard error (n = 3)

Table 1: Bioconcentration factors of *T. alexandrinum* for Cd, Pb, Cu and Zn

Metal	Metal concentration in soil (mg kg ⁻¹)			Bioconcentration factor of harvested tissues		
	Background concentration*	Concentration added to soil	Total concentration	BCF _{root}	BCF _{stem}	BCF _{leaves}
Cd	0.339	100	100.339	0.604	0.172	0.177
Pb	11.797	100	111.797	1.544	0.917	0.858
Cu	20.782	100	120.782	1.071	0.153	0.234
Zn	23.133	100	123.133	4.242	2.518	3.756

* Values of background soil concentrations of Cd, Pb, Cu and Zn were taken from a previous recent study (Sajad, 2010)

From Table 1 it is clear that the order of uptake of the heavy metals from the soil was: Zn > Pb > Cu > Cd (except BCF_{stem} in which case Cd > Cu). According to Jadia and Fulekar (2008), Zn is relatively mobile in soils and is the most abundant metal in roots and shoots of contaminated plants as it is in soils. Similarly, Ciura et al. (2005) stated that plants easily accumulate Zn in aboveground organs. According to Kumar et al. (2008), the mean concentrations of heavy metals in plants decreased according to the sequence: Zn > Cu > Pb > Ni > Co > Cd.

3.6 Translocation Factor

The values of translocation factor of *T. alexandrinum* for the selected heavy metals are given in Table 2.

Table 2: Translocation factors of *T. alexandrinum* for Cd, Pb, Cu and Zn

Metal	Metal concentration in soil (mg kg ⁻¹)			Translocation factor of shoots	
	Background concentration*	Concentration added to soil	Total concentration	TF _{stem}	TF _{leaves}
Cd	0.339	100	100.339	0.284	0.293
Pb	11.797	100	111.797	0.594	0.556
Cu	20.782	100	120.782	0.143	0.218
Zn	23.133	100	123.133	0.594	0.886

* Values of background soil concentrations of Cd, Pb, Cu and Zn were taken from a previous recent study (Sajad, 2010)

TF values follow the order: Zn ≥ Pb > Cd > Cu. All TF values are less than 1. According to Turan and Esringu (2007), the big difference between root and shoot concentrations indicates an important restriction of the internal transport of Cu, Cd, Pb, and Zn from roots to shoots resulting in higher root concentrations rather than translocation to shoots.

4. Conclusions

T. alexandrinum effectively extracted the selected heavy metals from the simulated heavy metal-contaminated soil as evident from the difference of heavy metal concentration values between control and experimental plants. This is also clear from BCF_{root} values, which are 4.242, 1.544, 1.071, and 0.604 for Zn, Pb, Cu, and Cd respectively. However, translocation of the accumulated heavy metals from roots to shoots was limited as seen from TF values for these metals, which are less than 1 in all cases. This is one of the limitations usually encountered in phytoremediation of toxic heavy metals. However, this limitation can be overcome by considering uprooting the plants in which case the accumulated heavy metals in the roots are removed from the soil. Using *T. alexandrinum* for phytoremediation has many advantages. For example, it produces considerable biomass, has a relatively short life cycle, is resistant to prevailing environmental and climatic conditions and above all offers multiple harvests in a single growth period. Thus, this candidate species can be used for phytoremediation of toxic heavy metals.

Acknowledgements

The authors are thankful to the Director, Centralized Resource Laboratory (CRL), University of Peshawar, for providing AAS facility.

5. References

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