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Heavy Metals Accumulation in Leaves and Tubers of Cassava (*Manihot Esculenta* Crantz) Grown in Crude Oil Contaminated Soil at Ikot Ada Udo, Nigeria

[1]HARRISON, UE; [1]OSU, SR; [2*]EKANEM, JO

[1]Department of Biology, College of Education, Afaha Nsit, Nigeria.
[2]Department of Microbiology, University of Uyo, Akwa Ibom State, Nigeria.
**Corresponding author Email: johnnykoko01@yahoo.com*

ABSTRACT: Pot experiment was set up to assess the levels of heavy metals accumulation in leaves and tubers of cassava (*Manihot esculenta* Crantz) grown in crude oil contaminated soil in the Niger Delta Region of Nigeria. Three cassava cultivars were used for the study viz: NR-8082, TMS-30572 and a Local variety (LV). The crops were planted in pots and nurtured to maturity. They were then assessed for the concentrations of the following heavy metals: Pb, Cd, Cr, Ni, Zn and Fe in both the leaves and tubers using standard laboratory procedures. Data analysis was carried out using the Analysis of Variance (ANOVA) and significantly different means were separated using the Duncan Multiple Range Test (DMRT). The analysis revealed that TMS accumulated the highest quantities of these metals, followed by NR and lastly, the local variety (V), this being a function of biomass production. Transfer Factors also proved the order of accumulation of this heavy metal as: TMS > NR > LV. Generally, the order of prevalence of heavy metal in both the leaves and the tubers was: Fe > Zn > Ni > Pb > Cd > Cr. This study revealed that crude oil pollution is increasingly raising the levels of heavy metals in the soils of Niger Delta and these metals are being taken up by plants including cassava, which is the most important staple food crop of the area. Consequently, crops farmers are advised against cultivating at crude oil contaminated sites for the risk of accumulation of heavy metals in plant tissues.

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Environmental pollution in the past few decades has become a topical issue. This is as a result of increase in oil exploration activities and related industrial development. (Osuji *et al.,* 2005). The Niger Delta area has been associated with frequent oil spills especially through oil pipe vandalization, tanker accidents and accidental rupture of oil pipelines. These mishaps result in the release of crude oil or refined petroleum products in to the terrestrial and aquatic environments (Okpokwasili and Amanchukwu, 1988). According to Agbogisi *et al*. (2005), Nigeria is a major producer and exporter of crude petroleum oil and continues to experience oil spills and this exposes the environment to hazards and its attendant effects on agricultural lands as well as on plant growth and development. It has been report that some heavy metals are associated with crude oil (Odokuma, 2009; Essiet *et al*., 2010). Essiett *et al.* (2010) discovered the elevated concentration of heavy metals in crude oil contaminated soils and reported that consuming plants growing in the vicinity of oil spills may pose a health risk to humans and animals.

Cassava (*Manihot esculenta* Crantz) plays an important role in terms of food Security, employment creation and income generation for farm families in most part of Akwa Ibom State and particularly to the people of Ikot Ada Udo Village in Ikot Abasi L.G.A., where these cassavas are abundantly cultivated. Cassava has become more than a staple food, sold fresh or processed into a storable form as a source of income. The people of Ikot Ada Udo usually process cassava into starch, tapioca, flour, garri, and fufu for domestic consumption and commercial purposes. Most at times, sweet cassava roots and leaves are fed to livestock. Tropical Manihot Species (TMS-30572), New Release (NR-8082) and Local Variety (Nsak Idaha) were commonly grown cassava cultivars in Ikot Ada Udo, Ikot Abasi L.G.A, but the local variety (Nsak Idaha) was more popularly grown than the other two varieties.

These cultivars are short day shrubs that can grow to a height of 1 to 4 meters respectively depending on the soil fertility and level of management. The growth habit of (TMS - 30572) and (NR-8082) are usually straight at the early growth, but branch profusely with good canopy at old age. Whereas the pattern of branching of the local variety (Nsak idaha) is always straight with little or no branches. Propagation in these cultivars are usually by stem cuttings. Other mophological attributes of the cultivars includes outer dark brown skin colour of root and creamy white inner

**Corresponding author Email: johnnykoko01@yahoo.com*

root skin. A mature tuber of three cultivars occasionally range in length from 12 to 25cm respectively.

Soil to plant transfer is one of the major pathways by which heavy metals and other contaminants in soils enter the food chain (Sparg *et al*., 2004). Food crops such as vegetables, tuber crops and cereals grown in crude oil impacted soil take up toxic metal from the soil (Harmanescu *et al*., 2011). Generally, most heavy metals are not biodegradable, have long biological half-lives and have the potential for accumulation in different body organs leading to unwanted side effects (Mbong *et al.,* 2014). Apau *et al.* (2014), posited that accumulation of heavy metals in crop plants is often of great concern due to its potential for food contamination through the soil root interface. Therefore, this research was designed to investigate heavy metal accumulation in leaves and tubers of cassava (*Manihot esculenta* Crantz) grown in crude oil contaminated soil in the Niger Delta Region of Nigeria.

MATERIALS AND METHOD

Description of Study Area: The study was carried out in an oil impacted farmland, located around the cork well at Ikot Ada Udo in Ikot Abasi L.G.A. situated within Niger Delta region of Nigeria. According to Udo (2008), Ikot Ada Udo is a village located in Ikot Abasi Local Government Area of Akwa Ibom State of Nigeria (Figure 1). It is situated within latitudes 04° 41'50'' and 04°41'48''N and longitude 07°41'06''E and 07°41'03''E. Mean annual temperature varies between 26 – 28^{0}C with relative humidity of 75 – 80%. The area is in the south-eastern agro-ecological zone of Nigeria.

The climate of the area is humid tropical. The rainfall is heavy, having a mean annual value close to 4000mm. The rainy season is from April to November and the dry season begins in November and ends in March. Harmattan occurs in the months of December and January. The soils in the area are formed on tertiary coastal plain sands (Petters, *et al.,* 1989).

The soils contain so many layers with loamy sand to sandy loam surface over clay loam to sandy clay subsoil. Because of their sandy nature, they are fragile and highly susceptible to erosion. They are also acidic and are generally referred to as 'Acid Sands' since they are both acidic and sandy. The vegetation in the area is tropical rain forest. However, in most of the areas the original rain forest has virtually disappeared because of clearing the forest for farming and lumbering.

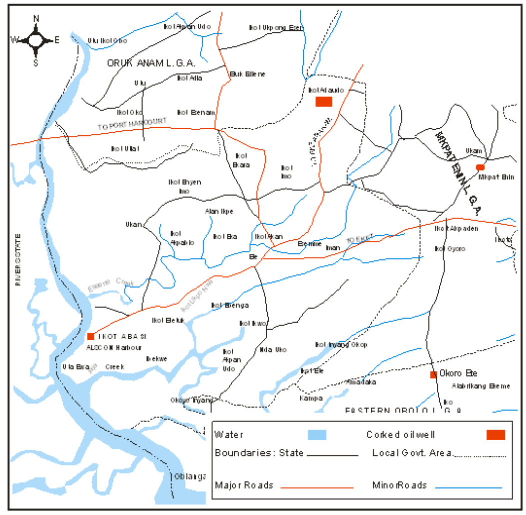

Fig 1: location of the corked oil well (Ibibio 1) at Ikot Ada Udo in Ikot Abasi Local Government Area. SOURCE: (Udoh, 2008)

Collection of Soil Samples from the Field for Laboratory Analysis: The Soil was tilled by mixing the soil and breaking the lumps. This was done using shovel. Soil samples were collected randomly with soil auger at the depth of 0 – 15 and 15 – 30 cm at five different locations and thoroughly mixed from which a composite sample was obtained (NRCRI, 1990). In the Laboratory, the samples were air-dried and made to pass through a 2mm mesh sieve and were analyzed for physical and chemical properties using standard methods (AOAC, 1990). The same procedures were repeated using un-impacted soil.

Preparation of Soil Sample for Laboratory Analysis: After sampling, the fresh soil samples were taken to laboratory in well labeled amber glasses. The soil samples were spread in drying trays and a dried for 48 hours in the laboratory. After drying, soil samples were mechanically crushed using a pestle and mortar and passed through a sieve to collect fine particles and the particles were stored for analysis while all the coarse soil particles were discarded (Lei *et al*., 2008).

*Soil Digestion***:** A gram of the remaining soil sample was weighed into a digestion flask and 10ml of perchloric acid added. Twenty ml HNO_3 was also added. The samples were digested using hot plate and sand bath until the colour of the digest changes to white desiccate. The sample was cooled and 50ml of distilled water was added to the digestion flask. The solution was filtered using a Whatman filter paper into a clean volumetric flask and stored for analysis.

Physical and Chemical Analysis of Soil Samples: Soil samples were analyzed following the standard procedures outlined by the American Public Health Association (APHA, 1998). Soil pH were measured using Beckman's glass electrode pH meter, organic carbon by the Walkey Black wet oxidation method (Jackson, 1962), available phosphorus by Bray P-1 method (Jackson, 1962). The total nitrogen content was determined by Micro-Kjeldahl method (Jackobson, 1992). Soil particle size distributions were determined by the hydrometer method (Udo and Ogunwale, 1986) using mechanical shaker, and sodiumhexametaphosphate as physical and chemical dispersant. Exchange acidity was determined by titration with 1N KCl (Kramprath, 1967).

Total exchangeable Bases were determined after extraction with 1M NH_4OAc (One molar ammonium acetate solution). Total exchangeable bases were determined by EDTA titration method while sodium and potassium were determined by photometry method. The Effective Cation Exchange Capacity (ECEC) was calculated by the summation method (that is summing up of the exchangeable bases and exchangeable acidity). Base saturation was calculated by dividing total exchangeable bases by ECEC multiplied by 100.

Cultivation of Cassava Cultivars (TMS - 30572, NR - 8082, Local Variety – Nsak Idaha): Three cassava cultivars (TMS 30572, NR 8082 and Local Variety-Nsak Idaha) were sown in the sack bag filled with 20kg contaminated soil. Viable cassava stems obtained from the three cultivars were cut into 20cm in length. To avoid termite infestation, the cuttings were treated with vetox 25 and allowed to dry before sowing i.e. 0.25kg in 180 litres of water (Udo *et al*., 2005). Two cuttings per perforated sack-bag of 50kg capacity, filled with oil impacted soil /un-impacted soil as control, were pinned on to the soil at an angle of 45^0 (Udo *et al*., 2005). Sprouting occurred two weeks after sowing. The plants were nurtured by watering at interval of two weeks and allowed to grow for 24 weeks before harvest.

Collections of Leaves and Tuber Samples from Cassava Cultivars: The Leaves and tubers of the three cassava cultivars were identified before harvest, from the experimental farm. The samples were collected in three replicates, stored in labeled polythene bags and taken to the Central Research Laboratory, University of Uyo. The samples were thoroughly washed with distilled water before cutting into smaller seizes. The samples were dried in the Galenkamp oven – preset at 60ºC for three days before milling. The milled samples were separately stored in air-tight containers for preservation and subsequent use for analysis.

Preparation of Leaves and Tubers for Laboratory Analysis: The Leaves and Tubers from the three cassava cultivars were air dried to remove the moisture and were then oven-dried using Galenkamp oven at a temperature of 65ºC to a constant weight. These were pulverized to fine powder using a laboratory grinder. According to Udo *et al*. (2009) three grams of each sample was weighed into clean platinum crucible and ashed at 450 - 500ºC, then cooled to room temperature in a desiccator. The ash was dissolved in 5 ml of 20 % hydrochloric acid and the solution was carefully transferred into a 100ml volumetric flask. The crucible was rinsed with distilled water, transferred to the flask, made up to the mark with distilled water and shaken to mix well. The resulting sample solutions were then taken for the determination of the heavy metal Lead (Pb), Cadmium (Cd), Chromium (Cr), Nickel (Ni), Zinc (Zn) and Iron (Fe) concentrations using Atomic Absorption Spectrophotometer (AAS).

Determination of Heavy Metals: 5g of each sample was digested in a glass tube with 5ml of concentrated nitric acid/ perchloric acid mixture (2:1) at 110ºC for two hours, diluted with 10ml of double deionized water and boiled at 100ºC for additional 2hrs, the digests were further diluted up to 25ml level with deionized water. Flame (air acetylene) atomic absorption spectrophotometry (Unicam 919model) was used to measure Pb, Cd, Cr, Ni, Zn and Fe concentrations in the plant's parts as heavy metals.

Estimation of Accumulation Factors / Transfer Factor (TF) for Heavy Metals: Accumulation factors (AF) was calculated using the equation:

$$AF = \frac{Concentration\ of\ heavy\ metals\ in\ plants}{Concentration\ of\ heavy\ metals\ in\ soil} \ldots\ldots \quad (1)$$

Transfer Factor is the ratio of the level of heavy metals or anions in a plant to the level of heavy metals or anions in the soil. The Transfer Factors (TF) for each heavy metal and anion were based on the method described by Harrison and Chirgawi, (1989). Metal levels and anion levels in the extracts of soils and plants were calculated on basis of dry weight according to Cui *et al*. (2005).

$$TF = \frac{Level\ of\ heavy\ metals\ in\ plants}{Level\ of\ heavy\ metals\ in\ soil} \quad (2)$$

Statistical Analysis: Data collected were subjected to statistical analysis such as Analysis of variance (ANOVA) and Standard Error Means (SEM). And

Dunkan Multiple Range Test (DMRT) were employed to separate means.

RESULTS AND DISCUSSION

Lead: Soil concentration of Pb was 1.37 mg kg-1 (Table 1). Concentration of lead in the leaves of TMS (0.5 mg kg-1) was significantly ($p \leq 0.05$) the highest, followed by that of NR (0.3 mg kg-1) while the Local Variety (LV) had the least significant foliar lead concentration (0.1 mg kg-1) (Table 2). Lead concentration of the tuber of NR (0.51 mg kg-1) and LV (0.45 mg kg-1) were statistically at par and were lower than that of TMS (0.74 mg kg-1) (Table 3). Transfer factors of Pb from soil to cassava leaves were 0.36, 0.22 and 0.07 for TMS, NR and LV, respectively (Fig. 2) while that of the tubers of TMS, NR and LC were 0.54, 0.37 and 0.33, respectively (Fig. 3). Pb Transfer factors of leaves were in the order of TMS > NR > LV while that of the tubers were TMS > NR = LV.

*Cadmium***:** Concentration of Cd in the soil was 1.4 mg kg-1(Table 1). Concentration of Cadmium in the leaves of TMS (0.14 mg kg-1) and NR (0.13 mg kg-1) were the same and significantly higher than that of LV (0.08 mg kg-1) (Table 2). Cadmium concentration in the cassava tubers for NR (0.13mg kg-1) and LV (0.12 mg kg-1) were statistically at par and significantly lower than that of TMS (0.16 mg kg-1) (Table 3). Transfer factors of Cd from soil to cassava leaves were 0.1, 0.09 and 0.06 for TMS, NR and LV, respectively (Fig. 2) while that of the tubers of TMS, NR and LV were 0.11, 0.09 and 0.09, respectively (Fig. 3). There was no significant ($p \leq 0.050$) difference between the transfer factors of Cd among the three cultivars for both the leaves and tubers. However, an apparent consideration shows TMS to have the highest transfer factor, followed by NR and lastly LV.

Chromium: Concentration of Cd in the soil was 0.06 mg kg-1(Table 1). Chromium concentration in the cassava leaves for TMS, NR and LV were 0.03, 0.02 and 0.02 mg kg-1, respectively (Table 2) while that of the tubers were 0.04, 0.02 and 0.01 mg kg-1, respectively (Table 3). Chromium concentrations in leaves and tubers of NR and LV were statistically equal and significantly lower than those of TMS. Transfer factors of Cr from soil to cassava leaves were 0.50, 0.33 and 0.33 for TMS, NR and LV, respectively (Figure 2) while that of the tubers were 0.67, 0.33 and 0.17, respectively (Figure 3). Pb Transfer Factors of leaves were in the order of TMS > NR = LV while that of the tubers were TMS > NR > LV.

*Nickel***:** Soil concentration of Ni was 14.87 mg kg-1 (Table 1).Nickel concentration in the cassava leaves for TMS, NR and LV were 3.0, 1.0 and 3.0 mg kg-1, respectively (Table 2) while that in the tubers were 7.2, 5.5 and 4.1 mg kg-1, respectively (Table 3). Nickel concentrations in leaves and tubers of NR and LV were statistically equal and significantly lower than those of TMS. Transfer factors of Ni from soil to cassava leaves were 0.20, 0.07 and 0.20 for TMS, NR and LV, respectively (Figure 2) while that of the tubers of TMS, NR and LV were 0.48, 0.37 and 0.28, respectively (Figure 3). Transfer Factors for the leaves were in the order of: TMS = LV > NR while that of tubers was TMS > NR > LV.

Table 1: Heavy Metals Concentrations in Crude Oil Contaminated Ultisols Soil

	Pb	Cd	Cr	Ni	Zn	Fe
Impacted Soil	1.37±1.78	1.4±0.91	0.06±0.91	14.87±0.80	42.09±1.04	580±150.90
Unimpacted soil	1.23±0.14	1.22±0.31	0.02±0.30	6.35±0.25	16.35±0.03	150±110.23
*Safety limit	85	0.8	0.5	35	100	2,000

*Source: WHO-Europe) and FAO/WHO (2001)

Table 2: Effect of Crude Oil Contamination Ultisols Soil on the Concentrations of Heavy Metals in Cassava Leaves

Cultivar	Pb	Cd	Cr	Ni	Zn	Fe
TMS 30572	0.5±0.2a	0.14±0.3a	0.03±0.01a	3.0±1.0a	15.0±4.0a	73.2±11a
NR - 8082	0.3±0.4b	0.13±0.4a	0.02±0.04b	1.0±0.5b	11.0±15	68.6±53a
Local Variety	0.1±0.1c	0.08±0.02b	0.02±0.01b	3.0±2.0a	7.0±0.12c	41.3±13b
*Safety limit	2.00	0.02	1.30	10.00	100	2.00

Means with the same alphabet along the column are not significantly ($p \leq 0.05$) different from each other. *Source: WHO 1996

Table 3: Effect of Crude Oil Contamination Ultisols Soil on the Concentrations of Heavy Metals in Cassava Tubers

Cultivar	Pb	Cd	Cr	Ni	Zn	Fe
TMS - 30572	0.74±0.05a	0.16±0.02a	0.04±0.02a	7.2±4.6a	22.1±20.0a	88.9±21a
NR - 8082	0.51±0.07b	0.13±0.02b	0.02±0.01b	5.5±2.7b	17.5±15.0b	79.5±26 a
Local Variety	0.45±0.04b	0.12±0.01b	0.01±0.01b	4.1±3.4b	12.7±10.0c	57.2±33b
*Safety limit	2.00	0.02	1.30	10.00	100	20.00

Means with the same alphabet along the column are not significantly ($p \leq 0.05$) different from each other, * Source: WHO 1996

Zinc: Concentration of Ni in the soil was 42.09 mg kg-1 (Table 1). Zinc concentration in the cassava leaves for TMS, NR and LV was 15.0, 11.0 and 7.0 mg kg-1, respectively (Table 2) while that in the tubers were 22.1, 17.5 and 12.7 mg kg-1, respectively (Table 3). Leaves and tubers Zinc concentrations of TMS were significantly ($p \leq 0.05$) the highest, followed by that of NR while LV was the least significant leaves and tubers Zinc concentrations. Transfer factors of Zn from soil to cassava leaves were 0.36, 0.26 and 0.17 for TMS, NR and LV, respectively (Figure 2) while that in the tubers in TMS, NR and LV were 0.53, 0.42 and 0.30 (Figure 3). Transfer factors of Zn for cassava leaves and tubers were in the order of: TMS > NR > LV.

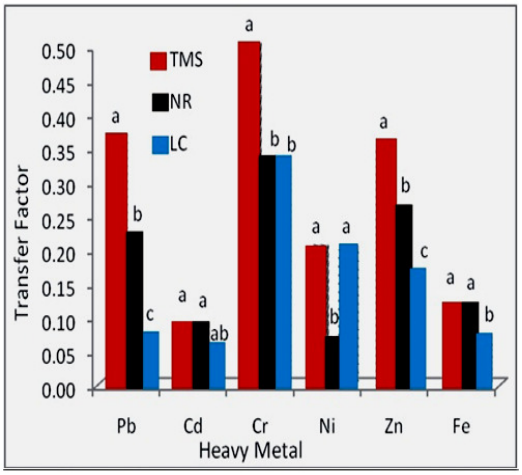

Fig 2: Transfer Factors for Heavy Metals in Leaves of Cassava Cultivars cultivatedin Crude Oil Contaminated Ultisol Soil

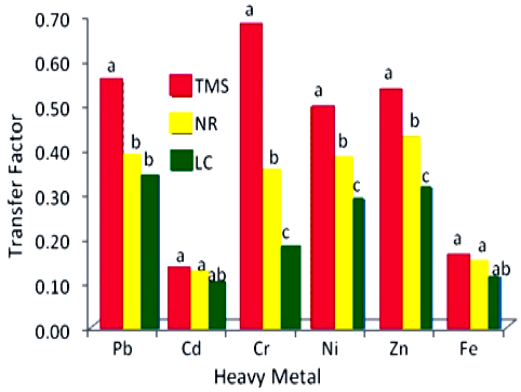

Fig 3: Transfer Factors for Heavy Metals in Tubers of Cassava Cultivars cultivated in Crude Oil Contaminated Ultisol Soil

Iron: Soil concentration of Fe was 580 mg kg-1(Table 1). Iron concentration in the cassava leaves for TMS, NR and LV were 73.2, 68.6 and 41.3 mg kg-1, respectively (Table 2) while that in the tubers were 88.9, 79.5 and 57.2 mg kg-1, respectively (Table 3). Leaves and tubers Fe concentrations of TMS and NR were statistically at par and were significantly ($p \leq 0.05$) higher than those of LV. Transfer factors of Fe from soil to cassava leaves were 0.13, 0.12 and 0.07 for TMS, NR and LV, respectively (Figure 2) while that in the tubers of TMS, NR and LC were 0.15, 0.14 and 0.10 (Figure 3). The trend of Transfer Factor for both leaves and tuber was: TMS = NR > LV. The mean concentration of heavy metals (Pb, Cd, Cr, Ni, Zn and Fe) showed that there was a higher concentrations of heavy metals in the crude oil impacted soils as compared to the un-impacted soil. According to Essiet *et al*. (2010) and Odokuma, 2009; elevation of heavy metal concentration is associated with crude oil contamination. This further supports earlier report of Ogbuechi *et al*. (2010) that the impact of crude oil on the Nigerian soil has deprived the soil of plant essential macro-nutrients but enriches the soil with heavy metals which are capable of posing health hazards to the inhabitant of the region. The concentration of heavy metals in tubers was higher than those in leaves. This is in agreement with the findings of Lichtfouse and Robert (2014) who reported that plant tubers accumulates more heavy metals than the leaves. The trend of heavy metals accumulation was predominantly in the order; TMS > NR > LV in the examined plant parts. Alloways (1993) reported that plant cultivar within a specie can differ widely in their ability to absorb, accumulate and tolerate heavy metals. In the study, TMS and NR (improved cultivars) were superior to the local variety (LV) in extracting heavy metals. The concentrations of heavy metals on these plant tissues were within safety limits (WHO, 1996; FAO/WHO, 2001) except for Cadmium (Cd) and Iron (Fe) which their concentrations were beyond the safety limits. The relatively higher Iron (Fe) concentration obtained from this study as the Centre for Disease Control and Prevention (CDCP) has warned that iron overload can lead to hemochromatosis, a disease characterized by fatigue, weakness, joint pain, abdominal pain or organ damage. Soil to plant transfer is one of the major pathways by which heavy metals and other contaminants in soil enter the food chain (Sparg *et al*., 2004; Mahmood and Malik, 2013). The high level of transfer factors (TF) recorded in this study is an indication of a possible transfer of heavy metals accumulated in the crude oil impacted soil into human system via consumption of products derived from the cassava plant obtained from this soil. Transfer factor (TF) is an essential tool for investigating the human health risk index (Cui *et al*., 2004).

Conclusion: Based on the findings of the study, there were higher levels of heavy metals in the crude oil impacted soil at ikot ada udo as compared to the unimpacted soil. The uptake of heavy metals differ with respect to the cassava cultivars as accumulation in tubers were higher than in leaves. It is thus recommended that multiple field experiments be carried out by a crop physiologist to confirm the findings. Essentially, crop farmers should be discouraged from cultivating on crude oil contaminated sites.

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